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(54) Title: STARCH MODIFICATION

(57) Abstract: The present invention relates to a method of altering starch synthesis in a plant by modifying the starch priming activity of the plant. In particular, this is achieved by altering the expression or activity of a starch primer which is preferably encoded by the sequence of SEQ ID NO: 1 or a sequence substantially homologous thereto. Also provided are plants in which the starch priming activity has been altered, and propagating material derived from such plants.

STARCH MODIFICATION

This invention is based upon the identification of a protein, which initiates starch synthesis in a plant. In particular, the intention relates to plant glycogenin-like nucleic acid molecules, plant glycogenin-like gene products, antibodies to plant glycogenin-like gene products, plant glycogenin-like regulatory regions, vectors and expression vectors with plant glycogenin-like genes, cells, plants and plant parts with plant glycogenin-like genes, modified starch from such plants and the use of the foregoing to improve agronomically valuable plants.

Starch, a branched polymer of glucose consisting of largely linear amylose and highly branched amylopectin, is the product of carbon fixation during photosynthesis in plants, and is the primary metabolic energy reserve stored in seeds and fruit. For example, up to 75% of the dry weight of grain in cereals is made up of starch. The importance of starch as a food source is reflected by the fact that two thirds of the world's food consumption (in terms of calories) is provided by the starch in grain crops such as wheat, rice and maize.

Starch is the product of photosynthesis, and is analogous to the storage compound glycogen in eukaryotes. It is produced in the chloroplasts or amyloplasts of plant cells, these being the plastids of photosynthetic cells and non-photosynthetic cells, respectively. The biochemical pathway leading to the production of starch in leaves has been well characterised, and considerable progress has also been made in elucidating the pathway of starch biosynthesis in storage tissues.

The biosynthesis of starch molecules is dependent on a complex interaction of numerous enzymes, including several essential enzymes such as ADP-Glucose, a series of starch synthases which use ADP glucose as a substrate for forming chains of glucose linked by alpha-1-4 linkages, and a series of starch branching enzymes that link sections of polymers with alpha-1-6 linkages to generate branched structures (Smith et al., 1995, *Plant Physiology*, 107:673-677). Further modification of the starch by yet other enzymes, i.e. debranching enzymes or disproportionating enzymes, can be specific to certain species.

The fine structure of starch is a complex mixture of D-glucose polymers that consist essentially of linear chains (amylose) and branched chains (amylopectin) glucans. Typically, amylose makes up between 10 and 25% of plant starch, but varies significantly among species. Amylose is composed of linear D-glucose chains typically 250-670 glucose units in

length (Tester, 1997, in: Starch Structure and Functionality, Frazier et al., eds., Royal Society of Chemistry, Cambridge, UK). The linear regions of amylopectin are composed of low molecular weight and high molecular weight chains, with the low ranging from 5 to 30 glucose units and the high molecular weight chains from 30 to 100 or more. The amylose/amylopectin ratio and the distribution of low and high molecular weight D-glucose chains can affect starch granule properties such as gelatinization temperature, retrogradation, and viscosity (Blanshard, 1987). The characteristics of the fine structure of starch mentioned above have been examined at length and are well known in the art of starch chemistry.

It is known that starch granule size and amylose percentage change during kernel development in maize and during tobacco leaf development (Boyer et al., 1976, Cereal Chem 53:327-337). In his classic study Boyer et al. concluded the amylose percentage of starch decreases with decreasing granule size in later stages of maize kernel development.

As mentioned above, glycogen serves as the glucose reserve in animals rather than starch. The biosynthesis of glycogen in eukaryotes involves chain elongation through the formation of linear alpha-1,4 glycosidic linkages catalysed by the enzyme, glycogen synthase. Evidence for a distinct initiation step involving a self-glucosylating protein, known as glycogenin or SGP, came from work directed at mammalian systems (Smythe et al., Eur. J. Biochem 200:625-631 (1990) and Whelan Bioessays 5:136-140 (1986)).

Cheng et al (Mol. and Cell Biol. 15(12): 6632-6640 (1995)) report the identification of two yeast genes whose products are implicated in the biosynthesis of glycogen. The two genes, *Glg1* and *Glg2* encode self-glucosylating proteins which *in vitro* act as primers for the elongation reaction catalysed by glycogen synthase. Disruption of both these genes results in the inability to synthesise glycogen, despite normal levels of glycogen synthase. Glycogenin homologues have been identified in *Caenorhabditis elegans* and humans (Mu et al., J. Biol. Chem. 272(44): 27589-27597(1997)).

It is now well established that glycogen synthesis is initiated on the primer protein, glycogenin or SGP, which remains covalently attached to the resulting macromolecule. The initiation step is thought to involve glycogenin growing a covalently attached oligosaccharide primer linked via a unique carbohydrate-protein bond via the hydroxyl group of the Tyr residue, Tyr 194. Once this oligosaccharide chain on glycogenin has been extended

sufficiently glycogen synthase is able to catalyse elongation and, together with the branching enzyme, form the mature glycogen molecule (Rodriguez and Whelan, Biochem Biophys Res Comm, 132:829-836; Roach and Skurat, 1997, in Progress in Nucleic Acid Research and Molecular Biology p289-316, Academic Press).

Previous workers have set out to determine whether a priming molecule, such as a self glucosylating protein, is responsible for the initiation of starch synthesis in plants. W094/04693 (Zeneca Ltd.) describes the purification of a putative starch priming protein molecule from maize endosperm, known as amylogenin, and isolation of a partial cDNA. The maize amylogenin showed no sequence homology with glycogenin and exhibited a novel glucose-protein bond (Singh *et al.*, FEBS Letters 376: 61-64 (1995)). However, based upon the sequence homology and the reported properties of the maize protein, it has subsequently been shown that the sequence of the maize nucleic acid molecule reported above is homologous to a reversibly glycosylated polypeptide (RGP1) from pea (Dhugga *et al.*, Proc. Natl. Acad. Sci. USA 94:7679-7684 (1997)). RGP1 is localised to the Golgi apparatus and is thought to be involved in cell wall synthesis. This has dispelled the initial idea that the "amylogenin" molecule of W094/04693 is involved in starch synthesis. In further work (Langeveld, M.J. S *et al.* 2002 Plant Physiol, 129, pp 278-289) it is concluded that wheat and rice RGPs do not play a role in starch synthesis in a way similar to the functioning of glycogenin as a primer for glycogen synthesis. It is reported that RGP1 and RGP2 proteins in wheat and rice have different functions to glycogenin.

Lightner *et al.* US 2002/0001843 described fragments of putative "corn (maize), wheat, and rice glycogenin and water stress proteins." Lightner *et al.* did not demonstrate the functionality of the fragments, but only their sequence homology to glycogenin from animals. To date, therefore, no one has identified and demonstrated a functional protein for starch initiation or starch priming in plants.

Purified starch is used in numerous food and industrial applications and is the major source of carbohydrates in the human diet. Typically, starch is mixed with water and cooked to form a thickening agent or gel. Of central importance are the temperature at which the starch cooks, the viscosity that the agent or gel reaches, and the stability of the gel viscosity over time. The physical properties of unmodified starch limit its usefulness in many

applications. As a result, considerable effort and expenditure is allocated to chemically modify starch (i.e. cross-linking and substitution) in order to overcome the numerous limitations of unmodified starch and to expand industrial usefulness. Modified starches can be used in foods, paper, textiles, and adhesives.

It is an object of the invention to provide novel isolated nucleic acid molecules and isolated polypeptides, which novel molecules and polypeptides are able to provide modified starch properties in transgenically modified plants.

The invention relates to a family of plant glycogenin-like genes, also referred to as starch primer genes. In various embodiments, the invention provides plant glycogenin-like nucleic acid molecules including, but not limited to, plant glycogenin-like genes; plant glycogenin-like regulatory regions; plant glycogenin-like promoters; and vectors incorporating sequences encoding plant glycogenin-like nucleic acid molecules of the invention. Also provided are plant glycogenin-like gene products, including, but not limited to, transcriptional products such as mRNAs, antisense and ribozyme molecules, and translational products such as the plant glycogenin-like protein, polypeptides, peptides and fusion proteins related thereto; genetically engineered host cells that contain any of the foregoing nucleic acid molecules and/or coding sequences or compliments, variants, or fragments thereof operatively associated with a regulatory element that directs the expression of the gene and/or coding sequences in the host cell; genetically-engineered plants derived from host cells; modified starch and starch granules produced by genetically-engineered host cells and plants; and the use of the foregoing to improve agronomically valuable plants.

In the context of the present invention, a "starch primer" used interchangeably with "plant glycogenin-like protein" includes any protein which is capable of initiating starch production in a plant. By definition, the plant glycogenin-like protein will be of plant origin. Preferred fragments of plant glycogenin-like proteins are those which retain the ability to initiate starch synthesis.

The invention is based upon the identification of a protein responsible for initiation of starch synthesis in plants, which despite continued efforts over the last few years, no one had yet successfully identified. In particular, the inventors have discovered nucleic acid molecules from *Arabidopsis* which have sequences that are homologous to the known

glycogenin genes of yeast and human. Analysis of one of this nucleic acid molecule indicates that it contains a sequence encoding a transit peptide for plastid localization of the gene product, consistent with a role in starch synthesis, referred to herein as plant glycogenin-like starch initiation protein (PGSIP). Glycogenin-like genes from other plant species have been identified by analysis of sequence homology with the *Arabidopsis* sequences. The genes of the invention do not show homology to the amylogenin sequences or starch sequences of the prior art.

Modulation of the initiation of starch synthesis allows various aspects of the biosynthetic process to be regulated. By altering aspects of the biosynthesis process such as temporal and spatial specificity, yield and storage, the carbohydrate profile of the plant may be altered in magnitude and directions that may be more favorable for nutritional or industrial uses.

The present invention provides an isolated nucleic acid molecule that i) comprises a nucleotide sequence which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: 3, or a fragment thereof; ii) comprises a nucleotide sequence at least 40% identical to SEQ ID NOs: 1 or 2, or a complement thereof as determined using the BESTFIT or GAP programs with a gap weight of 50 and a length weight of 3; or iii) hybridizes to a nucleic acid molecule consisting of SEQ ID NOs: 1 or 2 under low stringency conditions of hybridization of washing at 60°C for 2x 15 minutes at 2 x SSC, 0.5x SDS, or a complement thereof. The present invention also provides an isolated nucleic acid molecule of the invention comprising SEQ ID NOs: 1 or 2 or a complement thereof. In an embodiment of the invention, an isolated nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of nucleotide residues 516-592, 681-918, 1039-1655, 1762-2536 and 2991-3264 of SEQ ID NO: 1.

Another embodiment of the invention encompasses an isolated nucleic acid molecule of the invention that i) comprises a nucleotide sequence which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: 11, or a fragment thereof; ii) comprises a nucleotide sequence at least 70% identical to SEQ ID NO: 10, or a complement thereof as determined using the BESTFIT or GAP programs with a gap weight of 50 and a length weight of 3, wherein the nucleotide sequence does not encode an amino acid of SEQ ID NO:

35; or iii) hybridizes to a nucleic acid molecule consisting of SEQ ID NO: 10 under stringent conditions of hybridization, or a complement thereof, wherein the sequence does not encode an amino acid of SEQ ID NO: 35. In a related embodiment, the isolated nucleic acid molecule of the invention comprises SEQ ID NO: 10 or a complement thereof. In another related embodiment an isolated nucleic acid molecule of the invention comprises the amino acid sequence that is at least 98% identical to SEQ ID NO: 9 as determined using the BESTFIT or GAP programs with a gap weight of 12 and a length weight of 4. The invention also encompasses an isolated nucleic acid molecule that comprises the nucleotide sequence of SEQ ID NO: 8 or a complement thereof.

In an embodiment of the invention, an isolated nucleic acid molecule of the invention i) comprises a nucleotide sequence which encodes a polypeptide comprising the amino acid sequence of SEQ ID NOs: 7, 13, 15, 17, 19, 21, 22, 24, 26, 28, 30, 32, 34, or a fragment thereof; ii) comprises a nucleotide sequence at least 70% identical to SEQ ID NOs: 4, 5, 6, 12, 14, 16, 18, 20, 23, 25, 27, 29, 31, 33, or a complement thereof as determined using the BESTFIT or GAP programs with a gap weight of 50 and a length weight of 3; or iii) hybridizes to a nucleic acid molecule consisting of SEQ ID NOs: 4, 5, 6, 12, 14, 16, 18, 20, 23, 25, 27, 29, 31, 33 under stringent conditions of hybridization, or a complement thereof. In a related embodiment, the isolated nucleic acid molecule of the invention comprises SEQ ID NOs: 4, 5, 6, 12, 14, 16, 18, 20, 23, 25, 27, 29, 31, 33, or a complement thereof. In another embodiment of the invention, a fragment of the isolated nucleic acid molecule of the invention comprises at least 40, 60, 80, 100 or 150 contiguous nucleotides of the nucleic acid molecule. In yet another embodiment, the isolated nucleic acid molecule of the invention comprises the nucleotide sequence of nucleotides 1-195 of SEQ ID NO: 2, or a complement thereof.

According to one aspect of the invention, an isolated polypeptide of the invention comprises the amino acid sequence of amino acid residues 1-65 of SEQ ID NO: 3, or a fragment thereof. In a related aspect, an isolated polypeptide comprises i) an amino acid sequence that is at least 70% identical to SEQ ID NO: 3 or a fragment thereof as determined using the BESTFIT or GAP programs with a gap weight of 12 and a length weight of 4; ii) an amino acid sequence encoded by the nucleic acid molecule of the invention; or iii) an amino

acid sequence of SEQ ID NO: 3.

An embodiment of the invention encompasses an isolated polypeptide of the invention that comprises i) an amino acid sequence at least 70% identical to SEQ ID NO: 11 as determined using the BESTFIT or GAP programs with a gap weight of 12 and a length weight of 4, or a fragment thereof; ii) an amino acid sequence encoded by the nucleic acid molecule of the invention; or iii) an amino acid sequence of SEQ ID NO: 11.

In another embodiment of the invention, an isolated polypeptide of the invention comprises i) an amino acid sequence that is at least 98% identical to SEQ ID NO: 9 as determined using the BESTFIT or GAP programs with a gap weight of 12 and a length weight of 4; iii) an amino acid sequence encoded by the nucleic acid molecule of SEQ ID NO: 8, or a complement thereof; or v) an amino acid sequence of SEQ ID NO: 9, or a fragment thereof.

The invention further provides for an isolated polypeptide that comprises i) an amino acid sequence that is at least 70% identical to SEQ ID NOs: 7, 13, 15, 17, 19, 21, 22, 24, 26, 28, 30, 32, 34, or a fragment thereof as determined using the BESTFIT or GAP programs with a gap weight of 12 and a length weight of 4; ii) an amino acid sequence encoded by the nucleic acid molecule of the invention; or iii) an amino acid sequence of SEQ ID NOs: 7, 13, 15, 17, 19, 21, 22, 24, 26, 28, 30, 32, 34. In an embodiment of the invention, a fragment of a polypeptide of the invention comprises at least 5 amino acid residues, wherein said fragment is a portion of the polypeptide encoded by a nucleic acid molecule selected from the group consisting of exon I, exon II, exon III, exon IV and exon V of SEQ ID NO: 1.

Another embodiment of the invention encompasses the polypeptide of SEQ ID: 3, 7, 9, 11, 13, 15, 17, 19, 21, 22, 24, 26, 28, 30, 32, 34 further comprising one or more conservative amino acid substitution. In yet another embodiment, the invention provides for a fusion protein comprising the amino acid sequence of the invention and a heterologous protein.

The invention provides for an isolated polypeptide fragment or immunogenic fragment that comprises at least 5, 8, 10, 15, 20, 25, 30 or 35 consecutive amino acids of a polypeptide according to the invention. The invention further provides for an antibody that immunospecifically binds to a polypeptide of the invention.

In one embodiment the invention encompasses a method for making a polypeptide of any one of the invention, comprising the steps of a) culturing a cell comprising a recombinant polynucleotide encoding a polypeptide of the invention under conditions that allow said polypeptide to be expressed by said cell; and b) recovering the expressed polypeptide.

According to another aspect of the invention, the present invention provides a complex comprising a polypeptide encoded by a nucleic acid molecule of the invention and a starch molecule. In one embodiment of the complex of the invention, the starch molecule comprises from 1 to 700 glucose units. In another embodiment of the complex of the invention the starch molecule comprises branching chains of glucose polysaccharides.

According to yet another aspect of the invention, the present invention provides a vector comprises a nucleic acid molecule of the invention. Alternatively, the present invention provides an expression vector comprises a nucleic acid molecule of the invention and at least one regulatory region operably linked to the nucleic acid molecule.

Advantageously the expression vector of the invention comprises a regulatory region that confers chemically-inducible, dark-inducible, developmentally regulated, developmental-stage specific, wound-induced, environmental factor-regulated, organ-specific, cell-specific, and/or tissue-specific expression of the nucleic acid molecule or constitutive expression of the nucleic acid molecule of the invention. Advantageously the expression vector of the invention comprises a regulatory region selected from the group consisting of a 35S CaMV promoter, a rice actin promoter, a patatin promoter and a high molecular weight glutenin gene of wheat. In another embodiment, an expression vector of the invention comprises the antisense sequence of a nucleic acid molecule of the invention, wherein the antisense sequence is operably linked to at least one regulatory region.

The invention also provides for a genetically-engineered cell which comprises a nucleic acid molecule of the invention. In one embodiment, a cell comprises the expression vector of the invention comprising a nucleic acid molecule of the invention and at least one regulatory region operably linked to the nucleic acid molecule. In another embodiment, a cell comprises the expression vector of the invention comprising the antisense sequence of nucleic acid molecules of the invention, wherein the antisense sequence is operably linked to at least one regulatory region.

Yet another aspect of the invention provides a genetically-engineered plant comprising the isolated nucleic acid molecule of the invention. The invention also provides a genetically-engineered plant comprising an isolated nucleic acid molecule of the invention and progeny thereof, and further comprising a transgene encoding an antisense nucleotide sequence. The invention also provides for a genetically-engineered plant comprising an isolated nucleic acid molecule of the invention, and further comprising an RNA interference construct.

An embodiment of the invention encompasses a cell comprising a 35SCaMV constitutive promoter operably linked to a nucleic acid molecule of the invention, fragments thereof, or the nucleic acid molecule of SEQ ID NO:2 or a rice actin promoter operably linked to an RNA interference construct comprising a nucleic acid molecule of the invention, fragments thereof, or fragments of a nucleic acid molecule of SEQ ID NO:2.

Another aspect of the invention provides a method of altering starch synthesis in a plant comprising, introducing into a plant an expression vector of the invention, such that starch synthesis is altered relative to a plant without the expression vector. Yet another embodiment of the invention provides a method of altering starch synthesis in a plant comprising, introducing into a plant at least an expression vector comprising the antisense sequence of a nucleic acid molecule of the invention, wherein the antisense sequence is operably linked to at least one regulatory region, such that starch synthesis is altered in comparison to a plant without the expression vector.

In another aspect of the invention, the present invention provides a method of altering starch granules in a plant comprises introducing into a plant at least an expression vector comprising a nucleic acid molecule of the invention and at least one regulatory region operably linked to the nucleic acid molecule, such that the starch granules are altered in comparison to a plant without the expression vector.

Advantageously the present invention provides a method of altering starch granules in a plant comprises introducing into a plant at least an expression vector of Claim 30??check, such that the starch granules are altered in comparison to a plant without the expression vector.

The invention further provides a method of altering starch granules in a plant

comprises introducing into a plant at least an expression vector comprising a nucleic acid molecule of the invention and at least one regulatory region operably linked to the nucleic acid molecule, such that the starch granules are absent from leaves of the plant comprising at least an expression vector.

In a preferred embodiment of the invention, a plant part comprises a nucleic acid molecule of the invention resulting in an alteration in starch synthesis. In another preferred embodiment the plant part is a tuber, seed, or leaf.

The invention also provides for the modified starch obtained from the plant parts of the invention, wherein the modification is selected from the group consisting of a ratio of amylose to amylopectin, amylose content, size of starch granules, quantity of size of starch granules, a ratio of small to large starch granules, and rheological properties of the starch as measured using viscometric analysis.

The present invention will now be illustrated by way of non-limiting examples, with reference to the sequence identifiers and Figures in which:

SEQ ID NO:1 shows the genomic sequence of a starch primer gene isolated from *Arabidopsis thaliana* referred to herein as plant glycogenin-like starch initiation protein (PGSIP), at3g18660, GenBank Accession No. NM_112752. The gene includes part of the promoter region, where the putative TATA and CAAT box are located at nucleotides 424-428 and 373-376 respectively. The exons are located at nucleotides 516-592, 681-918, 1039-1655, 1762-2536 and 2991-3264.

SEQ ID NO: 2 shows the deduced cDNA sequence of *Arabidopsis thaliana* PGSIP with protein translation. The transit peptide is located at nucleotides 1-195.

SEQ ID NO:3 shows the amino acid sequence representing the *Arabidopsis thaliana* PGSIP protein. The predicted transit peptide is located at amino acid residues 1-65.

SEQ ID NO:4 shows the nucleotide sequence of the maize EST of GenBank Accession No. BF729544 with homology to the *Arabidopsis thaliana* PGSIP gene. The nucleotide sequence with homology to the *Arabidopsis thaliana* PGSIP gene is located at nucleotides 1-557.

SEQ ID NO:5 shows the nucleotide sequence of the maize EST BG837930 with homology to *Arabidopsis thaliana* PGSIP gene. The nucleotide sequence with homology to the *Arabidopsis thaliana* PGSIP gene is located at nucleotides 1-726.

SEQ ID NO:6 shows the deduced cDNA of the *Arabidopsis* glycogenin-like gene (at1g77130) with protein translation. The protein sequence with homology to a small region (amino acid residues 1023-1146) of dull1 gene from maize (064923).

SEQ ID NO:7 shows the amino acid sequence of at1g77130.

SEQ ID NO:8 shows the deduced cDNA of the *Arabidopsis* glycogenin-like gene (at1g08990) GenBank Accession No. NM_100770 with protein translation.

SEQ ID NO:9 shows the amino acid sequence of at1g08990.

SEQ ID NO:10 shows the deduced cDNA of the *Arabidopsis* glycogenin-like gene (at1g54940) GenBank Accession No. NM_104367 with protein translation.

SEQ ID NO:11 shows the amino acid sequence of at1g54940.

SEQ ID NO:12 shows the deduced cDNA of the *Arabidopsis* glycogenin-like gene (at4g33330) GenBank Accession No. NM_119487 with protein translation.

SEQ ID NO:13 shows the amino acid sequence of at4g33330.

SEQ ID NO:14 shows the deduced cDNA of the *Arabidopsis* glycogenin-like gene (at4g33340) GenBank Accession No. NM_119488 with protein translation.

SEQ ID NO:15 shows the amino acid sequence of at4g33340.

SEQ ID NO:16 shows the nucleotide sequence of Barley EST Seq1.

SEQ ID NO:17 shows the amino acid sequence of Barley EST Seq1.

SEQ ID NO:18 shows the nucleotide sequence of Barley EST Seq2.

SEQ ID NO:19 shows the amino acid sequence of Barley EST Seq2.

SEQ ID NO:20 shows the nucleotide sequence of a wheat EST.

SEQ ID NO:21 shows the first half of the amino acid sequence of the wheat EST.

SEQ ID NO:22 shows the second half of the amino acid sequence of the wheat EST.

SEQ ID NO:23 shows the deduced cDNA of the *Arabidopsis* gene EMBL:AY062695 GenBank Accession No. AY062695 with homology to the *Arabidopsis* PGSIP gene with protein translation.

SEQ ID NO:24 shows the amino acid sequence of EMBL:AY062695.

SEQ ID NO:25 shows the deduced cDNA of the Rice gene SPTrEMBL:Q94HG3 GenBank Accession No. AC079633 with homology to the *Arabidopsis* PGSIP gene with protein translation.

SEQ ID NO:26 shows the amino acid sequence of SPTrEMBL:Q94HG3.

SEQ ID NO:27 shows the nucleotide sequence of Maize EST Seq1.

SEQ ID NO:28 shows the amino acid sequence of Maize EST Seq1.

SEQ ID NO:29 shows the nucleotide sequence of Maize EST Seq2.

SEQ ID NO:30 shows the amino acid sequence of Maize EST Seq2.

SEQ ID NO:31 shows the nucleotide sequence of Maize EST Seq3.

SEQ ID NO:32 shows the amino acid sequence of Maize EST Seq3.

SEQ ID NO:33 shows the nucleotide sequence of Maize EST Seq4.

SEQ ID NO: 34 shows the amino acid sequence of Maize EST Seq4.

SEQ ID NO: 35 shows an amino acid sequence as a result of a conceptual translation of a portion of a genomic clone from *Arabidopsis thaliana* as it appears in US Patent Application No. 2002/0001843.

Figure 1 shows the plasmid containing the *Arabidopsis thaliana* plant glycogenin-like starch initiation protein (PGSIP) gene.

Figure 2 shows the plasmid map for pTPYES.

Figure 3 shows the plasmid map for pNTPYES

Figure 4A shows a genomic region containing AT3g18660 (PGSIP); 4B shows a non-radioactive southern blot of *Arabidopsis*, wheat and maize genomic DNA probed with C-terminus AT3g18660 cDNA under high stringency conditions. N-NcoI, A-AvaI, C-ClaI. The probe used for the blot of Figure 4B is also shown.

Figure 5A shows a non-radioactive southern blot of *Arabidopsis*, wheat and maize genomic DNA probed with N-terminal ATg18660 (PGSIP) cDNA fragment under low stringency conditions. N-NcoI, A-AvaI, C-ClaI. Lane M is a marker, lane 1 is AT (EcoRI), lane 2 is AT (XhoI), lane 3 is AT (EcoRV), lane 4 is wheat (EcoRI), lane 5 is wheat (XhoI), lane 6 is wheat EcoRV), lane 7 is maize (EcoRI), lane 8 is maize (XhoI), and lane 9 is maize (EcoRV); 5B shows a non-radioactive southern blot of *Arabidopsis*, wheat and maize genomic DNA probed with C-terminal ATg18660 (PGSIP) cDNA fragment under low stringency conditions. N-NcoI, A-AvaI, C-ClaI. Lane M is a marker, lane 1 is AT (EcoRI), lane 2 is AT (XhoI), lane 3 is AT (EcoRV), lane 4 is wheat (EcoRI), lane 5 is wheat (XhoI),

lane 6 is wheat EcoRV), lane 7 is maize (EcoRI), lane 8 is maize (XbaI), and lane 9 is maize (EcoRV): 5C shows the N-terminal and C-terminal region of the PGSIP cDNA used to probe the blots of 5A and 5B.

Figure 6 shows the cloning strategy and plasmid maps for the production of the PGSIP RNAi construct pCL76 SCV.

Figure 7 shows the plasmid map for pCL68 SCV. (Sense expression construct) containing the AT3g18660 (PGSIP) cDNA.

Figure 8 shows the plasmid map for pCL76 SCV.(RNAi construct) containing fragments of the AT3g18660 (PGSIP) cDNA.

Figure 9 shows the plasmid map for pMC177 (Sense expression construct) containing the AT3g18660 (PGSIP) under rice actin promoter used in barley and *Arabidopsis* transformation.

Figure 10 shows the plasmid map for pMC176 (RNAi construct) containing the AT3g18660 (PGSIP) under rice actin promoter used in barley and *Arabidopsis* transformation.

Figure 11A shows the results of iodine staining of leaves of barley which was shown to be PCR positive for the (pCL76 SCV) RNAi PGSIP constructs. Starch grains are absent; 11B shows the results of iodine staining of leaves of barley which was shown to be PCR negative for the (pCL76 SCV) RNAi PGSIP constructs. Starch grains are visible.

For purposes of clarity, and not by way of limitation, the invention is described in the subsections below in terms of (a) plant glycogenin-like nucleic acid molecules; (b) plant glycogenin-like gene products; (c) transgenic plants that ectopically express plant glycogenin-like protein; (d); transgenic plants in which endogenous plant glycogenin-like protein expression is suppressed; (e) starch characterized by altered structure and physical properties produced by the methods of the invention.

1.0 PLANT GLYCOGENIN-LIKE NUCLEIC ACIDS

The nucleic acid molecules of the invention may be DNA, RNA and comprises the nucleotide sequences of a plant glycogenin-like gene, or fragments or variants thereof. A polynucleotide is intended to include DNA molecules (e.g., cDNA, genomic DNA), RNA molecules (e.g., hnRNA, pre-mRNA, mRNA, double-stranded RNA), and DNA or RNA

analogs generated using nucleotide analogs. The polynucleotide can be single-stranded or double-stranded.

The nucleic acid molecules are characterized by their homology to known glycogen primer (glycogenin) genes, such as those from yeast (Glg1 and Glg2), human (any isoform), *C. elegans*, rat or rabbit, or plant glycogenin-like gene such as those defined herein. A preferred nucleic acid molecule of this embodiment is one that encodes the amino acid sequence of SEQ ID NO: 2, or a fragment or variant thereof, or a nucleic acid molecule comprising a sequence substantially similar to SEQ ID NO: 2. In a most preferred embodiment, the nucleic acid molecule comprises the nucleotide sequence shown in SEQ ID NO: 1, or a fragment or variant thereof, or a sequence substantially similar to SEQ ID NO: 1. The variants may be allelic variants. Allelic variants being multiple forms of a particular gene or protein encoded by a particular gene. Fragments of a plant glycogenin-like gene may include regulatory elements of the gene such as promoters, enhancers, transcription factor binding sites, and/or segments of a coding sequence for example, a conserved domain, exon, or transit peptide.

In a preferred embodiment, the nucleic acid molecules of the invention are comprised of full length sequences in that they encode an entire plant glycogenin-like protein as it occurs in nature. Examples of such sequences include SEQ ID NOs: 1, 2, 6, 8, 10, 12, and 14. The corresponding amino acid sequences of full length glycogenin-like proteins are SEQ ID NOs: 3, 7, 9, 11, 13, and 15.

In an alternative embodiment, the nucleic acid molecules of the invention comprise a nucleotide sequence of SEQ ID NOs: 1, 2, 4, 5, 6, 8, 10, 12, 14, 16, 18, 20, 23, 25, 27, 29, 31, or 33.

The nucleic acid molecules and their variants can be identified by several approaches including but not limited to analysis of sequence similarity and hybridization assays.

In the context of the present invention the term "substantially homologous," "substantially identical," or "substantial similarity," when used herein with respect to sequences of nucleic acid molecules, means that the sequence has either at least 45% sequence identity with the reference sequence, preferably 50% sequence identity, more preferably at least 60%, 70%, 80%, 90% and most preferably at least 95% sequence identity

with said sequences, in some cases the sequence identity may be 98% or more preferably 99%, or above, or the term means that the nucleic acid molecule is either is capable of hybridizing to the complement of the nucleic acid molecule having the reference sequence under stringent conditions.

"% identity", as known in the art, is a measure of the relationship between two polynucleotides or two polypeptides, as determined by comparing their sequences. In general, the two sequences to be compared are aligned to give a maximum correlation between the sequences. The alignment of the two sequences is examined and the number of positions giving an exact amino acid or nucleotide correspondence between the two sequences determined, divided by the total length of the alignment and multiplied by 100 to give a % identity figure. This % identity figure may be determined over the whole length of the sequences to be compared, which is particularly suitable for sequences of the same or very similar length and which are highly homologous, or over shorter defined lengths, which is more suitable for sequences of unequal length or which have a lower level of homology.

For example, sequences can be aligned with the software clustalw under Unix which generates a file with a ".aln" extension, this file can then be imported into the Bioedit program (Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids. Symp. Ser. 41:95-98) which opens the .aln file. In the Bioedit window, one can choose individual sequences (two at a time) and alignment them. This method allows for comparison of the entire sequences.

Methods for comparing the identity of two or more sequences are well known in the art. Thus for instance, programs available in the Wisconsin Sequence Analysis Package, version 9.1 (Devereux J *et al*, Nucleic Acids Res. 12:387-395, 1984, available from Genetics Computer Group, Maidson, Wisconsin, USA). The determination of percent identity between two sequences can be accomplished using a mathematical algorithm. For example, the programs BESTFIT and GAP, may be used to determine the % identity between two polynucleotides and the % identity between two polypeptide sequences. BESTFIT uses the "local homology" algorithm of Smith and Waterman (Advances in Applied Mathematics, 2:482-489, 1981) and finds the best single region of similarity between two sequences. BESTFIT is more suited to comparing two polynucleotide or two polypeptide sequences

which are dissimilar in length, the program assuming that the shorter sequence represents a portion of the longer. In comparison, GAP aligns two sequences finding a "maximum similarity" according to the algorithm of Neddleman and Wunsch (J. Mol. Biol. 48:443-354, 1970). GAP is more suited to comparing sequences which are approximately the same length and an alignment is expected over the entire length. Preferably the parameters "Gap Weight" and "Length Weight" used in each program are 50 and 3 for polynucleotides and 12 and 4 for polypeptides, respectively. Preferably % identities and similarities are determined when the two sequences being compared are optimally aligned.

Other programs for determining identity and/or similarity between sequences are also known in the art, for instance the BLAST family of programs (Karlin & Altschul, 1990, *Proc. Natl. Acad. Sci. USA* 87:2264-2268, modified as in Karlin & Altschul, 1993, *Proc. Natl. Acad. Sci. USA* 90:5873-5877, available from the National Center for Biotechnology Information (NCBI), Bethesda, Maryland, USA and accessible through the home page of the NCBI at www.ncbi.nlm.nih.gov). These programs exemplify a preferred, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences. Such an algorithm is incorporated into the BLASTN and BLASTX programs of Altschul, et al., 1990, *J. Mol. Biol.* 215:403-410. BLAST nucleotide searches can be performed with the BLASTN program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to a nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to a protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., 1997, *Nucleic Acids Res.* 25:3389-3402. Alternatively, PSI-Blast can be used to perform an iterated search which detects distant relationships between molecules (*Id.*). When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (e.g., BLASTX and BLASTN) can be used. See <http://www.ncbi.nlm.nih.gov>. Another preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, 1988, *CABIOS* 4:11-17. Such an algorithm is incorporated into the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid

sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

Another non-limiting example of a program for determining identity and/or similarity between sequences known in the art is FASTA (Pearson W.R. and Lipman D.J., Proc. Nat. Acad. Sci., USA, 85:2444-2448, 1988, available as part of the Wisconsin Sequence Analysis Package). Preferably the BLOSUM62 amino acid substitution matrix (Henikoff S. and Henikoff J.G., Proc. Nat. Acad. Sci., USA, 89:10915-10919, 1992) is used in polypeptide sequence comparisons including where nucleotide sequences are first translated into amino acid sequences before comparison.

Yet another non-limiting example of a program known in the art for determining identity and/or similarity between amino acid sequences is SeqWeb Software (a web-based interface to the GCG Wisconsin Package: Gap program) which is utilized with the default algorithm and parameter settings of the program: blosum62, gap weight 8, length weight 2.

The percent identity between two sequences can be determined using techniques similar to those described above, with or without allowing gaps. In calculating percent identity, typically exact matches are counted.

Preferably the program BESTFIT is used to determine the % identity of a query polynucleotide or a polypeptide sequence with respect to a polynucleotide or a polypeptide sequence of the present invention, the query and the reference sequence being optimally aligned and the parameters of the program set at the default value.

Alternatively, variants and fragments of the nucleic acid molecules of the invention can be identified by hybridization to SEQ ID NOS: 1, 2, 4-6, 8, 10, 12, 14, 16, 18, 20, 23, 25, 27, 29, 31, or 33. In the context of the present invention "stringent conditions" are defined as those given in Martin *et al* (EMBO J 4:1625-1630 (1985)) and Davies *et al* (Methods in Molecular Biology Vol 28: Protocols for nucleic acid analysis by non-radioactive probes, Isaac, P.G. (ed), Humana Press Inc., Totowa N.J, USA)). Hybridization was carried out overnight at 65°C (high stringency conditions) or 55°C (low stringency conditions). The filters were washed for 2 x 15 minutes with 0.1 x SSC, 0.5 x SDS at 65°C (high stringency washing). For low

stringency washing, the filters were washed at 60°C for 2x 15 minutes at 2 x SSC, 0.5x SDS.

In instances wherein the nucleic acid molecules are oligonucleotides ("oligos"), highly stringent conditions may refer, *e.g.*, to washing in 6xSSC / 0.05% sodium pyrophosphate at 37°C (for 14-base oligos), 48°C (for 17-base oligos), 55°C (for 20-base oligos), and 60°C (for 23-base oligos). These nucleic acid molecules may act as plant glycogenin-like gene antisense molecules, useful, for example, in plant glycogenin-like gene regulation and/or as antisense primers in amplification reactions of plant glycogenin-like gene and/or nucleic acid molecules. Further, such nucleic acid molecules may be used as part of ribozyme and/or triple helix sequences, also useful for plant glycogenin-like gene regulation. Still further, such molecules may be used as components in probing methods whereby the presence of a plant glycogenin-like allele may be detected.

In one embodiment, a nucleic acid molecule of the invention may be used to identify other plant glycogenin-like genes by identifying homologs. This procedure may be performed using standard techniques known in the art, for example screening of a cDNA library by probing; amplification of candidate nucleic acid molecules; complementation analysis, and yeast two-hybrid system (Fields and Song *Nature* 340 245-246 (1989); Green and Hannah *Plant Cell* 10 1295-1306 (1998)).

The invention also includes nucleic acid molecules, preferably DNA molecules, that are amplified using the polymerase chain reaction and that encode a gene product functionally equivalent to a plant glycogenin-like gene product.

In another embodiment of the invention, nucleic acid molecules which hybridize under stringent conditions to the nucleic acid molecules comprising a plant glycogenin-like gene and its complement are used in altering starch synthesis in a plant. Such nucleic acid molecules may hybridize to any part of a plant glycogenin-like gene, including the regulatory elements. Preferred nucleic acid molecules are those which hybridize under stringent conditions to a nucleic acid molecule comprising the nucleotide sequence encoding the amino acid sequence of SE ID NO: 2, and/or a nucleotide sequence of any one of SEQ ID NOs: 1, 2, 4-6, 8, 10, 12, 14, 16, 18, 20, 23, 25, 27, 29, 31, or 33 or their complement sequences. Preferably the nucleic acid molecule which hybridizes under stringent conditions to a nucleic acid molecule comprising the sequence of a plant glycogenin-like gene or its complement are

complementary to the nucleic acid molecule to which they hybridize.

In another embodiment of the invention, nucleic acid molecules which hybridize under stringent conditions to the nucleic acid molecules of SEQ ID NOs: 1, 2, 4-6, 8, 10, 12, 14, 16, 18, 20, 23, 25, 27, 29, 31, or 33 hybridize over the full length of the sequences of the nucleic acid molecules.

Alternatively, nucleic acid molecules of the invention or their expression products may be used in screening for agents which alter the activity of a plant glycogenin-like protein of a plant. Such a screen will typically comprise contacting a putative agent with a nucleic acid molecule of the invention or expression product thereof and monitoring the reaction there between. The reaction may be monitored by expression of a reporter gene operably linked to a nucleic acid molecule of the invention, or by binding assays which will be known to persons skilled in the art.

Fragments of a plant glycogenin-like nucleic acid molecule of the invention preferably comprise or consist of at least 40 continuous or consecutive nucleotides of the plant glycogenin-like nucleic acid molecule of the invention, more preferably at least 60 nucleotides, at least 80 nucleotides, or most preferably at least 100 or 150 nucleotides in length. Fragments of a plant glycogenin-like nucleic acid molecule of the invention encompassed by the invention may include elements involved in regulating expression of the gene or may encode functional plant glycogenin-like proteins. Fragments of the nucleic acid molecules of the invention, encompasses fragments of SEQ ID NOs: 1, 2, 4-6, 8, 10, 12, 14, 16, 18, 20, 23, 25, 27, 29, 31 and 33 as well as fragments of the variants of those sequences identified as defined above by percent homology or hybridization.

Examples of fragments encompassed by the invention include exons of the PGSIP gene. SEQ ID NO: 1 indicates exon and intron boundaries of the plant glycogenin-like gene PGSIP. Nucleic acid molecules comprising PGSIP exon and intron sequences are encompassed by the present invention. In one embodiment, five exons are included (SEQ ID NO:1; GenBank Accession No. NM_112752). PGSIP exon 1 encompasses nucleotides 516-592 of SEQ ID NO: 1. of the sequence shown in SEQ ID NO:1; exon 2 encompasses nucleotides 681 to 918 of the sequence shown in SEQ ID NO:1; exon 3 encompasses nucleotides 1039 to 1655 of the sequence shown in SEQ ID NO:1; exon 4 encompasses

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nucleotides 1762 to 2536 of the sequence shown in SEQ ID NO:1; exon 5 encompasses nucleotides 2991 to 3264 of the sequence shown in SEQ ID NO:1.

Further, a plant glycogenin-like nucleic acid molecule of the invention can comprise two or more of any above-described sequences, or variants thereof, linked together to form a larger subsequence.

The nucleic acid molecules of the invention can comprise or consist of an EST sequence. The EST nucleic acid molecules of the invention can be used as probes for cloning corresponding full length genes. For example, the barley EST of SEQ ID NO: 16 can be utilized as a probe in identifying and cloning the full length Barley homolog of the *Arabidopsis* PGSIP gene. The EST nucleic acid molecules of the invention may be used as sequence probes in connection with computer software to search databases, such as GenBank for homologous sequences. Alternatively, the EST nucleic acid molecules can be used as probes in hybridization reactions as described herein. The EST nucleic acid molecules of the invention can also be used as molecular markers to map chromosome regions.

In certain embodiments, the plant glycogenin-like nucleic acid molecules and polypeptides do not include sequences consisting of those sequences known in the art. For example, in one embodiment, the plant glycogenin-like nucleic acid molecules do not include EST sequences.

In other embodiments, the plant glycogenin-like nucleic acid molecules of the invention, encode polypeptides that function as plant glycogenin-like proteins. The functionality of such nucleic acid molecules can be assessed using the yeast hybrid complementation assay as described herein in Example 3. Alternatively, the functionality of such nucleic acid molecules can be assessed using a complementation assay in *Arabidopsis* as described in this section.

An isolated nucleic acid molecule encoding a variant protein can be created by introducing one or more nucleotide substitutions, additions or deletions into the plant glycogenin-like nucleic acid molecule, such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations can be introduced by standard techniques, such as, ethyl methane sulfonate, X-rays, gamma rays, T-DNA mutagenesis, or site-directed mutagenesis, PCR-mediated mutagenesis. Briefly, PCR primers

are designed that delete the trinucleotide codon of the amino acid to be changed and replace it with the trinucleotide codon of the amino acid to be included. This primer is used in the PCR amplification of DNA encoding the protein of interest. This fragment is then isolated and inserted into the full length cDNA encoding the protein of interest and expressed recombinantly.

An isolated nucleic acid molecule encoding a variant protein can be created by any of the methods described in section 1.1. Either conservative or non-conservative amino acid substitutions can be made at one or more amino acid residues. Both conservative and non-conservative substitutions can be made. Conservative replacements are those that take place within a family of amino acids that are related in their side chains. Genetically encoded amino acids are can be divided into four families: (1) acidic = aspartate, glutamate; (2) basic = lysine, arginine, histidine; (3) nonpolar = alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; and (4) uncharged polar = glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine. In similar fashion, the amino acid repertoire can be grouped as (1) acidic = aspartate, glutamate; (2) basic = lysine, arginine histidine, (3) aliphatic = glycine, alanine, valine, leucine, isoleucine, serine, threonine, with serine and threonine optionally be grouped separately as aliphatic-hydroxyl; (4) aromatic = phenylalanine, tyrosine, tryptophan; (5) amide = asparagine, glutamine; and (6) sulfur-containing = cysteine and methionine. (See, for example, Biochemistry, 4th ed., Ed. by L. Stryer, WH Freeman and Co.: 1995).

Alternatively, mutations can be introduced randomly along all or part of the coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for biological activity to identify mutants that retain activity. Following mutagenesis, the encoded protein can be expressed recombinantly and the activity of the protein can be determined.

The invention also encompasses (a) DNA vectors that contain any of the foregoing nucleic acids and/or coding sequences (i.e. fragments and variants) and/or their complements (i.e., antisense molecules); (b) DNA expression vectors that contain any of the foregoing nucleic acids and/or coding sequences operatively associated with a regulatory region that directs the expression of the nucleic acids and/or coding sequences; and (c) genetically

engineered host cells that contain any of the foregoing nucleic acids and/or coding sequences operatively associated with a regulatory region that directs the expression of the gene and/or coding sequences in the host cell. As used herein, regulatory region include, but are not limited to, inducible and non-inducible genetic elements known to those skilled in the art that drive and regulate expression of a nucleic acid. The nucleic acid molecules of the invention may be under the control of a promoter, enhancer, operator, cis-acting sequences, or trans-acting factors, or other regulatory sequence. The nucleic acid molecules encoding regulatory regions of the invention may also be functional fragments of a promoter or enhancer. The nucleic acid molecules encoding a regulatory region is preferably one which will target expression to desired cells, tissues, or developmental stages.

Examples of highly suitable nucleic acid molecules encoding regulatory regions are endosperm specific promoters, such as that of the high molecular weight glutenin (HMWG) gene of wheat, prolamin, or ITR1, or other suitable promoters available to the skilled person such as gliadin, branching enzyme, ADFG pyrophosphorylase, patatin, starch synthase, rice actin, and actin, for example.

Other suitable promoters include the stem organ specific promoter gSPO-A, the seed specific promoters Napin, KTI 1, 2, & 3, beta-conglycinin, beta-phaseolin, heliathin, phytohemagglutinin, legumin, zein, lectin, leghemoglobin c3, ABI3, PvAlf, SH-EP, EP-C1, 2S1, EM 1, and ROM2.

Constitutive promoters, such as CaMV promoters, including CaMV 35S and CaMV 19S may also be suitable. Other examples of constitutive promoters include Actin 1, Ubiquitin 1, and HMG2.

In addition, the regulatory region of the invention may be one which is environmental factor-regulated such as promoters that respond to heat, cold, mechanical stress, light, ultra-violet light, drought, salt and pathogen attack. The regulatory region of the invention may also be one which is a hormone-regulated promoter that induces gene expression in response to phytohormones at different stages of plant growth. Useful inducible promoters include, but are not limited to, the promoters of ribulose bisphosphate carboxylase (RUBISCO) genes, chlorophyll a/b binding protein (CAB) genes, heat shock genes, the defense responsive gene (e.g., phenylalanine ammonia lyase genes), wound induced genes (e.g., hydroxyproline rich

cell wall protein genes), chemically-inducible genes (e.g., nitrate reductase genes, gluconase genes, chitinase genes, PR-1 genes etc.), dark-inducible genes (e.g., asparagine synthetase gene as described by U.S. Patent 5,256,558), and developmental-stage specific genes (e.g., Shoot Meristemless gene, ABI3 promoter and the 2S1 and Em 1 promoters for seed development (Devic et al., 1996, *Plant Journal* 9(2):205-215), and the kin1 and cor6.6 promoters for seed development (Wang et al., 1995, *Plant Molecular Biology*, 28(4):619-634). Examples of other inducible promoters and developmental-stage specific promoters can be found in Datla et al., in particular in Table 1 of that publication (Datla et al., 1997, *Biotechnology annual review* 3:269-296).

A vector of the invention may also contain a sequence encoding a transit peptide which can be fused in-frame such that it is expressed as a fusion protein.

Methods which are well known to those skilled in the art can be used to construct vectors and/or expression vectors containing plant glycogenin-like protein coding sequences and appropriate transcriptional/translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques and *in vivo* recombination/genetic recombination. See, for example, the techniques described in Sambrook et al., 1989, and Ausubel et al., 1989. Alternatively, RNA capable of encoding plant glycogenin-like protein sequences may be chemically synthesized using, for example, synthesizers. See, for example, the techniques described in Gait, 1984, *Oligonucleotide Synthesis*, IRL Press, Oxford. In a preferred embodiment of the invention, the techniques described in Example 6, and illustrated in Figure 6 are used to construct a vector.

A variety of host-expression vector systems may be utilized to express the plant glycogenin-like gene products of the invention. Such host-expression systems represent vehicles by which the plant glycogenin-like gene products of interest may be produced and subsequently recovered and/or purified from the culture or plant (using purification methods well known to those skilled in the art), but also represent cells which may, when transformed or transfected with the appropriate nucleic acid molecules, exhibit the plant glycogenin-like protein of the invention *in situ*. These include but are not limited to microorganisms such as bacteria (e.g., *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing plant glycogenin-like protein coding

sequences; yeast (e.g., *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing the plant glycogenin-like protein coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing the plant glycogenin-like protein coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV); plant cell systems transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing plant glycogenin-like protein coding sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter; the cytomegalovirus promoter/enhancer; etc.). In a preferred embodiment of the invention, an expression vector comprising a plant glycogenin-like nucleic acid molecule operably linked to at least one suitable regulatory sequence is incorporated into a plant by one of the methods described in this section, section 1.3, 1.4 and 1.5 or in Examples 7, 8, 9, and 12.

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the plant glycogenin-like protein being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of antibodies or to screen peptide libraries, for example, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al., 1983, *EMBO J.* 2:1791), in which the plant glycogenin-like coding sequence may be ligated individually into the vector in frame with the *lac Z* coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, 1985, *Nucleic Acids Res.* 13:3101-9; Van Heeke & Schuster, 1989, *J. Biol. Chem.* 264:5503-9); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene protein can be released from the GST moiety.

In one such embodiment of a bacterial system, full length cDNA nucleic acid molecules are appended with in-frame Bam HI sites at the amino terminus and Eco RI sites at the carboxyl terminus using standard PCR methodologies (Innis et al., 1990, *supra*) and ligated into the pGEX-2TK vector (Pharmacia, Uppsala, Sweden). The resulting cDNA construct contains a kinase recognition site at the amino terminus for radioactive labeling and glutathione S-transferase sequences at the carboxyl terminus for affinity purification (Nilsson, et al., 1985, *EMBO J.* 4:1075; Zabeau and Stanley, 1982, *EMBO J.* 1: 1217).

The recombinant constructs of the present invention may include a selectable marker for propagation of the construct. For example, a construct to be propagated in bacteria preferably contains an antibiotic resistance gene, such as one that confers resistance to kanamycin, tetracycline, streptomycin, or chloramphenicol. Examples of other suitable marker genes include antibiotic resistance genes such as those conferring resistance to G4 18 and hygromycin (*npt-II*, *hyg-B*); herbicide resistance genes such as those conferring resistance to phosphinothricin and sulfonamide based herbicides (*bar* and *suI* respectively; EP-A-242246, EP-A- 0369637) and screenable markers such as beta-glucuronidase (GB2 197653), luciferase and green fluorescent protein. Suitable vectors for propagating the construct include, but are not limited to, plasmids, cosmids, bacteriophages or viruses.

The marker gene is preferably controlled by a second promoter which allows expression in cells other than the seed, thus allowing selection of cells or tissue containing the marker at any stage of development of the plant. Preferred second promoters are the promoter of nopaline synthase gene of *Agrobacterium* and the promoter derived from the gene which encodes the 35S subunit of cauliflower mosaic virus (CaMV) coat protein. However, any other suitable second promoter may be used.

The nucleic acid molecule encoding a plant glycogenin-like protein may be native or foreign to the plant into which it is introduced. One of the effects of introducing a nucleic acid molecule encoding a plant glycogenin-like gene into a plant is to increase the amount of plant glycogenin-like protein present and therefore the amount of starch produced by increasing the copy number of the nucleic acid molecule. Foreign plant glycogenin-like nucleic acid molecules may in addition have different temporal and/or spatial specificity for starch synthesis compared to the native plant glycogenin-like protein of the plant, and so may

be useful in altering when and where or what type of starch is produced. Regulatory elements of the plant glycogenin-like genes may also be used in altering starch synthesis in a plant, for example by replacing the native regulatory elements in the plant or providing additional control mechanisms. The regulatory regions of the invention may confer expression of a plant glycogenin-like gene product in a chemically-inducible, dark-inducible, developmentally regulated, developmental-stage specific, wound-induced, environmental factor-regulated, organ-specific, cell-specific, tissue-specific, or constitutive manner. Alternatively, the expression conferred by a regulatory region may encompass more than one type of expression selected from the group consisting of chemically-inducible, dark-inducible, developmentally regulated, developmental-stage specific, wound-induced, environmental factor-regulated, organ-specific, cell-specific, tissue-specific, and constitutive.

Further, any of the nucleic acid molecules (including EST clone nucleic acid molecules) and/or polypeptides and proteins described herein, can be used as markers for qualitative trait loci in breeding programs for crop plants. To this end, the nucleic acid molecules, including, but not limited to, full length plant glycogenin-like genes coding sequences, and/or partial sequences (ESTs), can be used in hybridization and/or DNA amplification assays to identify the endogenous plant glycogenin-like genes, plant glycogenin-like gene mutant alleles and/or plant glycogenin-like gene expression products in cultivars as compared to wild-type plants. They can also be used as markers for linkage analysis of qualitative trait loci. It is also possible that the plant glycogenin-like genes may encode a product responsible for a qualitative trait that is desirable in a crop breeding program. Alternatively, the plant glycogenin-like protein and/or peptides can be used as diagnostic reagents in immunoassays to detect expression of the plant glycogenin-like genes in cultivars and wild-type plants.

Genetically-engineered plants containing constructs comprising the plant glycogenin-like nucleic acid and a reporter gene can be generated using the methods described herein for each plant glycogenin-like nucleic acid gene variant, to screen for loss-of-function variants induced by mutations, including but not limited to, deletions, point mutations, rearrangements, translocation, etc. The constructs can encode for fusion proteins comprising a plant glycogenin-like protein fused to a protein product encoded by a reporter gene.

Alternatively, the constructs can encode for a plant glycogenin-like protein and a reporter gene product that are not fused. The constructs may be transformed into the homozygous recessive plant glycogenin-like gene mutant background, and the restorative phenotype examined, i.e. quantity and quality of starch, as a complementation test to confirm the functionality of the variants isolated.

1.1 PLANT GLYCOGENIN-LIKE GENE PRODUCTS

The invention encompasses the polypeptides of SEQ ID Nos: 3, 7, 11, 13, 15, 17, 19, 21, 22, 24, 26, 28, 30, 31, 32, or 34. Plant glycogenin-like proteins, polypeptides and peptide fragments, variants, allelic variants, mutated, truncated or deleted forms of plant glycogenin-like proteins and/or plant glycogenin-like fusion proteins can be prepared for a variety of uses, including, but not limited to, the generation of antibodies, as reagents in assays, the identification of other cellular gene products involved in starch synthesis and/or starch synthesis initiation, etc.

Plant glycogenin-like translational products include, but are not limited to those proteins and polypeptides encoded by the sequences of the plant glycogenin-like nucleic acid molecules of the invention. The invention encompasses proteins that are functionally equivalent to the plant glycogenin-like gene products of the invention.

The primary use of the plant glycogenin-like gene products of the invention is to alter starch synthesis via increasing the number of priming or initiation sites for elongation of glucose chains.

In an embodiment of the invention, an isolated polypeptide comprises the amino acid molecule of SEQ ID NO: 9 or a variant or fragment thereof, provided the polypeptide sequence is not that of SEQ ID NO: 35.

The present invention also provides variants of the polypeptides of the invention. Such variants have an altered amino acid sequence which can function as either agonists (mimetics) or as antagonists. Variants can be generated by mutagenesis, *e.g.*, discrete point mutation or truncation. An agonist can retain substantially the same, or a subset, of the biological activities of the naturally occurring form of the protein. An antagonist of a protein can inhibit one or more of the activities of the naturally occurring form of the protein by, for

example, deleting one or more of the receiver domains. Thus, specific biological effects can be elicited by addition of a variant of limited function.

Modification of the structure of the subject polypeptides can be for such purposes as enhancing efficacy, stability, or post-translational modifications (*e.g.*, to alter the phosphorylation pattern of the protein). Such modified peptides, when designed to retain at least one activity of the naturally-occurring form of the protein, or to produce specific antagonists thereof, are considered functional equivalents of the polypeptides. Such modified peptides can be produced, for instance, by amino acid substitution, deletion, or addition.

For example, it is reasonable to expect that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid (*i.e.* isosteric and/or isoelectric mutations) will not have a major effect on the biological activity of the resulting molecule.

Whether a change in the amino acid sequence of a peptide results in a functional homolog (*e.g.*, functional in the sense that the resulting polypeptide mimics or antagonizes the wild-type form) can be readily determined by assessing the ability of the variant peptide to produce a response in cells in a fashion similar to the wild-type protein, or competitively inhibit such a response. Polypeptides in which more than one replacement has taken place can readily be tested in the same manner.

In a preferred embodiment, a mutant polypeptide that is a variant of a polypeptide of the invention can be assayed for: (1) the ability to complement glycogenin function in a yeast or plant system in which the native glycogenin or plant glycogenin-like genes have been knocked out; (2) the ability to form a complex with a glucose or oligosaccharide; or (3) the ability to promote initiation of elongation of polysaccharide chains.

The invention encompasses functionally equivalent mutant plant glycogenin-like proteins and polypeptides. The invention also encompasses mutant plant glycogenin-like proteins and polypeptides that are not functionally equivalent to the gene products. Such a mutant plant glycogenin-like protein or polypeptide may contain one or more deletions, additions or substitutions of plant glycogenin-like amino acid residues within the amino acid sequence encoded by any one of the plant glycogenin-like nucleic acid molecules described

above in Section 1.1, and which result in loss of one or more functions of the plant glycogenin-like protein, thus producing a plant glycogenin-like gene product not functionally equivalent to the wild-type plant glycogenin-like protein.

Plant glycogenin-like proteins and polypeptides bearing mutations can be made to plant glycogenin-like DNA (using techniques discussed above as well as those well known to one of skill in the art) and the resulting mutant plant glycogenin-like proteins tested for activity. Mutants can be isolated that display increased function, (e.g., resulting in improved root formation), or decreased function (e.g., resulting in suboptimal root function). In particular, mutated plant glycogenin-like proteins in which any of the exons shown in SEQ ID NO: 1 are deleted or mutated are within the scope of the invention. Additionally, peptides corresponding to one or more exons of the plant glycogenin-like protein, truncated or deleted plant glycogenin-like protein are also within the scope of the invention. Fusion proteins in which the full length plant glycogenin-like protein or a plant glycogenin-like polypeptide or peptide fused to an unrelated protein are also within the scope of the invention and can be designed on the basis of the plant glycogenin-like nucleotide and plant glycogenin-like amino acid sequences disclosed herein.

While the plant glycogenin-like polypeptides and peptides can be chemically synthesized (e.g., see Creighton, 1983, *Proteins: Structures and Molecular Principles*, W.H. Freeman & Co., NY) large polypeptides derived from plant glycogenin-like gene and the full length plant glycogenin-like gene may advantageously be produced by recombinant DNA technology using techniques well known to those skilled in the art for expressing nucleic acid molecules.

Nucleotides encoding fusion proteins may include, but are not limited to, nucleotides encoding full length plant glycogenin-like proteins, truncated plant glycogenin-like proteins, or peptide fragments of plant glycogenin-like proteins fused to an unrelated protein or peptide, such as for example, an enzyme, fluorescent protein, or luminescent protein that can be used as a marker or an epitope that facilitates affinity-based purification. Alternatively, the fusion protein can further comprise a heterologous protein such as a transit peptide or fluorescence protein.

In an embodiment of the invention, the percent identity between two polypeptides of

the invention is at least 40%. In a preferred embodiment of the invention, the percent identity between two polypeptides of the invention is at least 50%. In another embodiment, the percent the percent identity between two polypeptides of the invention is at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, or at least 98%. Determining whether two sequences are substantially similar may be carried out using any methodologies known to one skilled in the art, preferably using computer assisted analysis as described in section 1.1.

Further, it may be desirable to include additional DNA sequences in the protein expression constructs. Examples of additional DNA sequences include, but are not limited to, those encoding: a 3' untranslated region; a transcription termination and polyadenylation signal; an intron; a signal peptide (which facilitates the secretion of the protein); or a transit peptide (which targets the protein to a particular cellular compartment such as the nucleus, chloroplast, mitochondria or vacuole). The nucleic acid molecules of the invention will preferably comprise a nucleic acid molecule encoding a transit peptide, to ensure delivery of any expressed protein to the plastid. Preferably the transit peptide will be selective for plastids such as amyloplasts or chloroplasts, and can be native to the nucleic acid molecule of the invention or derived from known plastid sequences, such as those from the small subunit of the ribulose bisphosphate carboxylase enzyme (ssu of rubisco) from pea, maize or sunflower for example. Transit peptide comprising amino acid residues 1-65 of SEQ ID NO: 2 is an example of a transit peptide native to the polypeptide of the invention. Where an agonist or antagonist which modulates activity of the plant glycogenin-like protein is a polypeptide, the polypeptide itself must be appropriately targeted to the plastids, for example by the presence of plastid targeting signal at the N terminal end of the protein (Castro Silva Filho *et al* Plant Mol Biol 30 769-780 (1996) or by protein-protein interaction (Schenke PC *et al*, Plant Physiol 122 235-241 (2000) and Schenke *et al* PNAS 98(2) 765-770 (2001). The transit peptides of the invention are used to target transportation of plant glycogenin-like proteins as well as agonists or antagonists thereof to plastids, the sites of starch synthesis, thus altering the starch synthesis process and resulting starch characteristics.

The plant glycogenin-like proteins and transit peptides associated with the plant glycogenin-like genes of the present invention have a number of important agricultural uses. The transit peptides associated with the plant glycogenin-like genes of the invention may be

used, for example, in transportation of desired heterologous gene products to a root, a root modified through evolution, tuber, stem, a stem modified through evolution, seed, and/or endosperm of transgenic plants transformed with such constructs.

The invention encompasses methods of screening for agents (i.e., proteins, small molecules, peptides) capable of altering the activity of a plant glycogenin-like protein in a plant. Variants of a protein of the invention which function as either agonists (mimetics) or as antagonists can be identified by screening combinatorial libraries of mutants, e.g., truncation mutants, of the protein of the invention for agonist or antagonist activity. In one embodiment, a variegated library of variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into nucleic acid molecules such that a degenerate set of potential protein sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (e.g., for phage display). There are a variety of methods which can be used to produce libraries of potential variants of the polypeptides of the invention from a degenerate oligonucleotide sequence. Methods for synthesizing degenerate oligonucleotides are known in the art (see, e.g., Narang, 1983, *Tetrahedron* 39:3; Itakura et al., 1984, *Annu. Rev. Biochem.* 53:323; Itakura et al., 1984, *Science* 198:1056; Ike et al., 1983, *Nucleic Acid Res.* 11:477).

In addition, libraries of fragments of the coding sequence of a polypeptide of the invention can be used to generate a variegated population of polypeptides for screening and subsequent selection of variants. For example, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of the coding sequence of interest with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA which can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library can be derived which encodes N-terminal and internal fragments of various sizes of the protein of interest.

Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. The most widely used techniques, which are amenable to high through-put analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a technique which enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify variants of a protein of the invention (Arkin and Yourvan, 1992, *Proc. Natl. Acad. Sci. USA* 89:7811-7815; Delgrave et al., 1993, *Protein Engineering* 6(3):327-331).

An isolated polypeptide of the invention, or a fragment thereof, can be used as an immunogen to generate antibodies using standard techniques for polyclonal and monoclonal antibody preparation. The full-length polypeptide or protein can be used or, alternatively, the invention provides antigenic peptide fragments for use as immunogens. In one embodiment, the antigenic peptide of a protein of the invention or fragments or immunogenic fragments of a protein of the invention comprise at least 8 (preferably 10, 15, 20, 30 or 35) consecutive amino acid residues of the amino acid sequence of SEQ ID NO: 3, 7, 9, 11, 13, 15, 17, 19, 21, 22, 24, 26, 28, 30, 32, or 34 and encompasses an epitope of the protein such that an antibody raised against the peptide forms a specific immune complex with the protein.

Exemplary amino acid sequences of the polypeptides of the invention can be used to generate antibodies against plant glycogenin-like genes. In one embodiment, the immunogenic polypeptide is conjugated to keyhole limpet hemocyanin ("KLH") and injected into rabbits. Rabbit IgG polyclonal antibodies can purified, for example, on a peptide affinity column. The antibodies can then be used to bind to and identify the polypeptides of the invention that have been extracted and separated via gel electrophoresis or other means.

One aspect of the invention pertains to isolated plant glycogenin-like polypeptides of the invention, variants thereof, as well as variants suitable for use as immunogens to raise antibodies directed against a plant glycogenin-like polypeptide of the invention. In one

embodiment, the native polypeptide can be isolated, using standard protein purification techniques, from cells or tissues expressing a plant glycogenin-like polypeptide. In a preferred embodiment, plant glycogenin-like polypeptides of the invention are produced from expression vectors by recombinant DNA techniques. In another preferred embodiment, a polypeptide of the invention is synthesized chemically using standard peptide synthesis techniques.

An isolated or purified protein or biologically active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the protein is derived, or substantially free of chemical precursors or other chemicals when chemically synthesized. The language "substantially free" indicates protein preparations in which the protein is separated from cellular components of the cells from which it is isolated or recombinantly produced. Thus, protein that is substantially free of cellular material includes protein preparations having less than 20%, 10%, or 5% (by dry weight) of a contaminating protein. Similarly, when an isolated plant glycogenin-like polypeptide of the invention is recombinantly produced, it is substantially free of culture medium. When the plant glycogenin-like polypeptide is produced by chemical synthesis, it is preferably substantially free of chemical precursors or other chemicals.

Biologically active portions of a polypeptide of the invention include polypeptides comprising amino acid sequences identical to or derived from the amino acid sequence of the protein, such that the variants sequences comprise conservative substitutions or truncations (e.g., amino acid sequences comprising fewer amino acids than those shown in any of SEQ ID NOS: 3, 7, 9, 11, 13, 15, 17, 19, 21, 22, 24, 26, 28, 30, 32, and 34, but which maintain a high degree of homology to the remaining amino acid sequence). Typically, biologically active portions comprise a domain or motif with at least one activity of the corresponding protein. Domains or motifs include, but are not limited to, a biologically active portion of a protein of the invention can be a polypeptide which is, for example, at least 10, 25, 50, 100, 200, 300, 400 or 500 amino acids in length. Polypeptides of the invention can comprise, for example, a glycosylation domain or site for complexing with polysaccharide or for attachment of disaccharide or a monomeric unit thereof, or a site that interacts with starch synthase and other enzymes that act on the polysaccharide.

1.2 PRODUCTION OF TRANSGENIC PLANTS AND PLANT CELLS

The invention also encompasses transgenic or genetically-engineered plants, and progeny thereof. As used herein, a transgenic or genetically-engineered plant refers to a plant and a portion of its progeny which comprises a nucleic acid molecule which is not native to the initial parent plant. The introduced nucleic acid molecule may originate from the same species e.g., if the desired result is over-expression of the endogenous gene, or from a different species. A transgenic or genetically-engineered plant may be easily identified by a person skilled in the art by comparing the genetic material from a non-transformed plant, and a plant produced by a method of the present invention for example, a transgenic plant may comprise multiple copies of plant glycogenin-like genes, and/or foreign nucleic acid molecules. Transgenic plants are readily distinguishable from non-transgenic plants by standard techniques. For example a PCR test may be used to demonstrate the presence or absence of introduced genetic material. Transgenic plants may also be distinguished from non-transgenic plants at the DNA level by Southern blot or at the RNA level by Northern blot or at the protein level by western blot, by measurement of enzyme activity or by starch composition or properties.

The nucleic acids of the invention may be introduced into a cell by any suitable means. Preferred means include use of a disarmed Ti-plasmid vector carried by *Agrobacterium* by procedures known in the art, for example as described in EP-A-01 16718 and EP-A-0270822. *Agrobacterium* mediated transformation methods are now available for monocots, for example as described in EP 0672752 and WO00/63398. Alternatively, the nucleic acid may be introduced directly into plant cells using a particle gun. A further method would be to transform a plant protoplast, which involves first removing the cell wall and introducing the nucleic acid molecule and then reforming the cell wall. The transformed cell can then be grown into a plant.

In an embodiment of the present invention, *Agrobacterium* is employed to introduce the gene constructs into plants. Such transformations preferably use binary *Agrobacterium* T-DNA vectors (Bevan, 1984, *Nuc. Acid Res.* 12:8711-21), and the co-cultivation procedure (Horsch et al., 1985, *Science* 227:1229-31). Generally, the *Agrobacterium* transformation

system is used to engineer dicotyledonous plants (Bevan et al., 1982, *Ann. Rev. Genet.* 16:357-84; Rogers et al., 1986, *Methods Enzymol.* 118:627-41). The *Agrobacterium* transformation system may also be used to transform, as well as transfer, DNA to monocotyledonous plants and plant cells (see Hernalsteen et al., 1984, *EMBO J.* 3:3039-41; Hooykass-Van Slogteren et al., 1984, *Nature* 311:763-4; Grimsley et al., 1987, *Nature* 325:1677-79; Boulton et al., 1989, *Plant Mol. Biol.* 12:31-40.; Gould et al., 1991, *Plant Physiol.* 95:426-34).

Various alternative methods for introducing recombinant nucleic acid constructs into plants and plant cells may also be utilized. These other methods are particularly useful where the target is a monocotyledonous plant or plant cell. Alternative gene transfer and transformation methods include, but are not limited to, protoplast transformation through calcium-, polyethylene glycol (PEG)- or electroporation-mediated uptake of naked DNA (see Paszkowski et al., 1984, *EMBO J.* 3:2717-22; Potrykus et al., 1985, *Mol. Gen. Genet.* 199:169-177; Fromm et al., 1985, *Proc. Natl. Acad. Sci. USA* 82:5824-8; Shimamoto, 1989, *Nature* 338:274-6), and electroporation of plant tissues (D'Halluin et al., 1992, *Plant Cell* 4:1495-1505). Additional methods for plant cell transformation include microinjection, silicon carbide mediated DNA uptake (Kaepller et al., 1990, *Plant Cell Reporter* 9:415-8), and microprojectile bombardment (Klein et al., 1988, *Proc. Natl. Acad. Sci. USA* 85:4305-9; Gordon-Kamm et al., 1990, *Plant Cell* 2:603-18).

According to the present invention, desired plants and plant cells may be obtained by engineering the gene constructs described herein into a variety of plant cell types, including, but not limited to, protoplasts, tissue culture cells, tissue and organ explants, pollen, embryos as well as whole plants. In an embodiment of the present invention, the engineered plant material is selected or screened for transformants (*i.e.*, those that have incorporated or integrated the introduced gene construct or constructs) following the approaches and methods described below. An isolated transformant may then be regenerated into a plant. Alternatively, the engineered plant material may be regenerated into a plant, or plantlet, before subjecting the derived plant, or plantlet, to selection or screening for the marker gene traits. Procedures for regenerating plants from plant cells, tissues or organs, either before or after selecting or screening for marker gene or genes, are well known to those skilled in the

art.

A transformed plant cell, callus, tissue or plant may be identified and isolated by selecting or screening the engineered plant material for traits encoded by the marker genes present on the transforming DNA. For instance, selection may be performed by growing the engineered plant material on media containing inhibitory amounts of the antibiotic or herbicide to which the transforming marker gene construct confers resistance. Further, transformed plants and plant cells may also be identified by screening for the activities of any visible marker genes (e.g., the β -glucuronidase, luciferase, green fluorescent protein, B or C1 anythocyanin genes) that may be present on the recombinant nucleic acid constructs of the present invention. Such selection and screening methodologies are well known to those skilled in the art.

The present invention is applicable to all plants which produce or store starch. Examples of such plants are cereals such as maize, wheat, rice, sorghum, barley; fruit producing species such as banana, apple, tomato or pear; root crops such as cassava, potato, yam, beet or turnip; oilseed crops such as rapeseed, canola, sunflower, oil palm, coconut, linseed or groundnut; meal crops such as soya, bean or pea; and any other suitable species.

In a preferred embodiment of the present invention, the method comprises the additional step of growing the plant and harvesting the starch from a plant part. In order to harvest the starch, it is preferred that the plant is grown until plant parts containing starch develop, which may then be removed. In a further preferred embodiment, the propagating material from the plant may be removed, for example the seeds. The plant part can be an organ such as a stem, root, leaf, or reproductive body. Alternatively, the plant part may be a modified organ such as a tuber, or the plant part is a tissue such as endosperm.

1.3 TRANSGENIC PLANTS THAT ECTOPICALLY EXPRESS PLANT GLYCOGENIN-LIKE PROTEIN

According to one aspect of the invention, a nucleic acid molecule according to the invention is expressed in the plant cell, plant, or part of a plant that comprises a nucleotide sequence encoding a plant glycogenin-like protein, fragment of variant thereof. The nucleic

acid molecule expressed in the plant cell can comprise a nucleotide sequence encoding a full length plant glycogenin-like protein. Examples of such sequences include SEQ ID NOS: 1, 2, 6, 8, 10, 12, and 14, or variants thereof and the corresponding the amino acid sequences of SEQ ID NOS: 3, 7, 9, 11, 13, and 15 or variants thereof.

In an embodiment of the invention, the nucleic acid molecules of the invention are expressed in a plant cell and are transcribed only in the sense orientation. A plant that expresses a recombinant plant glycogenin-like nucleic acid may be engineered by transforming a plant cell with a nucleic acid construct comprising a regulatory region operably associated with a nucleic acid molecule, the sequence of which encodes a plant glycogenin-like protein or a fragment thereof. In plants derived from such cells, starch synthesis is altered in ways described in section 1.6. The term "operably associated" is used herein to mean that transcription controlled by the associated regulatory region would produce a functional mRNA, whose translation would produce the plant glycogenin-like protein. Starch may be altered in particular parts of a plant, including but not limited to seeds, tubers, leaves, roots and stems or modifications thereof.

In an embodiment of the invention, a plant is engineered to constitutively express a plant glycogenin-like protein in order to alter the starch content of the plant. In a preferred embodiment, the starch content is 40%, 30%, 20%, 10%, 5%, 2% greater than that of a non-engineered control plant(s). In another preferred embodiment, the starch content is 40%, 30%, 20%, 10%, 5%, 2% less than that of a non-engineered control plant(s).

In another aspect of the invention, where the nucleic acid molecules of the invention are expressed in a plant cell and are transcribed only in the sense orientation, the starch content of the plant cell and plants derived from such a cells exhibit altered starch content. The altered starch content comprises an increase in the ratio of amylose to amylopectin. In one embodiment of the invention, the ratio of amylose to amylopectin increases by 2%, 5%, 10%, 20%, 30%, 40%, or 50% in comparison to a non-engineered control plant(s).

In preferred embodiment of the invention, the nucleic acid molecules of the invention are expressed in a potato plant and are transcribed only in the sense orientation. The starch content of the plant, including the tubers, exhibit increased starch content. If the number of copies of the nucleic acid molecules of the invention are expressed in a potato plant that are

transcribed only in the sense orientation is increased, the starch content of the plant, including the tubers, increases.

In yet another embodiment of the present invention, it may be advantageous to transform a plant with a nucleic acid construct operably linking a modified or artificial promoter to a nucleic acid molecule having a sequence encoding a plant glycogenin-like protein or a fragment thereof. Such promoters typically have unique expression patterns and/or expression levels not found in natural promoters because they are constructed by recombining structural elements from different promoters. *See, e.g.,* Salina et al., 1992, *Plant Cell* 4:1485-93, for examples of artificial promoters constructed from combining cis-regulatory elements with a promoter core.

In a preferred embodiment of the present invention, the associated promoter is a strong root and/or embryo-specific plant promoter such that the plant glycogenin-like protein is overexpressed in the transgenic plant.

In yet another preferred embodiment of the present invention, the overexpression of plant glycogenin-like protein in starch producing organs and organelles may be engineered by increasing the copy number of the plant glycogenin-like gene. One approach to producing such transgenic plants is to transform with nucleic acid constructs that contain multiple copies of the complete plant glycogenin-like gene with native or heterologous promoters. Another approach is repeatedly transform successive generations of a plant line with one or more copies of the complete plant glycogenin-like gene constructs. Yet another approach is to place a complete plant glycogenin-like gene in a nucleic acid construct containing an amplification-selectable marker (ASM) gene such as the glutamine synthetase or dihydrofolate reductase gene. Cells transformed with such constructs is subjected to culturing regimes that select cell lines with increased copies of complete plant glycogenin-like gene. *See, e.g.,* Donn et al., 1984, *J. Mol. Appl. Genet.* 2:549-62, for a selection protocol used to isolate of a plant cell line containing amplified copies of the GS gene. Cell lines with amplified copies of the plant glycogenin-like gene can then be regenerated into transgenic plants.

1.4 TRANSGENIC PLANTS THAT SUPPRESS ENDOGENOUS PLANT GLYCOGENIN-LIKE PROTEIN EXPRESSION

The nucleic acid molecules of the invention may also be used to augment the starch priming activity of a plant cell, plant, or part of a plant, or alternatively to alter activity of the plant glycogenin-like protein of a plant cell, plant, or part of a plant by modifying transcription or translation of the plant glycogenin-like gene. In an embodiment of the invention, an antagonist which is capable of altering the expression of a nucleic acid molecule of the invention is introduced into a plant in order to alter the synthesis of starch. The antagonist may be protein, nucleic acid, chemical antagonist, or any other suitable moiety. In an embodiment of the invention, an antagonist which is capable of altering the expression of a nucleic acid molecule of the invention is provided to alter the synthesis of starch. The antagonist may be protein, nucleic acid, chemical antagonist, or any other suitable moiety. Typically, the antagonist will function by inhibiting or enhancing transcription from the plant glycogenin-like gene, either by affecting regulation of the promoter or the transcription process; inhibiting or enhancing translation of any RNA product of the plant glycogenin-like gene; inhibiting or enhancing the activity of the plant glycogenin-like protein itself or inhibiting or enhancing the protein-protein interaction of the plant glycogenin-like protein and downstream enzymes of the starch biosynthesis pathway. For example, where the antagonist is a protein it may interfere with transcription factor binding to the plant glycogenin-like gene promoter, mimic the activity of a transcription factor, compete with or mimic the plant glycogenin-like protein, or interfere with translation of the plant glycogenin-like RNA, interfere with the interaction of the plant glycogenin-like protein and downstream enzymes. Antagonists which are nucleic acids may encode proteins described above, or may be transposons which interfere with expression of the plant glycogenin-like gene.

The suppression may be engineered by transforming a plant with a nucleic acid construct encoding an antisense RNA or ribozyme complementary to a segment or the whole of plant glycogenin-like gene RNA transcript, including the mature target mRNA. In another embodiment, plant glycogenin-like gene suppression may be engineered by transforming a plant cell with a nucleic acid construct encoding a ribozyme that cleaves the plant

glycogenin-like gene mRNA transcript.

In another embodiment, the plant glycogenin-like mRNA transcript can be suppressed through the use of RNA interference, referred to herein as RNAi. RNAi allows for selective knock out of a target gene in a highly effective and specific manner. The RNAi technique involves introducing into a cell double-stranded RNA (dsRNA) which corresponds to exon portions of a target gene such as an endogenous plant glycogenin-like gene. The dsRNA causes the rapid destruction of the target gene's messenger RNA, i.e. an endogenous plant glycogenin-like gene mRNA, thus preventing the production of the plant glycogenin-like protein encoded by that gene. The RNAi constructs of the invention confer expression of dsRNA which correspond to exon portions of an endogenous plant glycogenin-like gene. The strands of RNA that form the dsRNA are complimentary strands from encoded by coding region, i.e., exons encoding sequence, on the 3' end of the plant glycogenin-like gene.

The dsRNA has an effect on the stability of the mRNA. The mechanism of how dsRNA results in the loss of the targeted homologous mRNA is still not well understood (Cogoni and Macino, 2000, Genes Dev 10: 638-643; Guru, 2000, Nature 404, 804-808; Hammond et al., 2001, Nature Rev Gen 2: 110-119). Current theories suggest a catalytic or amplification process occurs that involves initiation step and an effector step.

In the initiation step, input dsRNA is digested into 21-23 nucleotide "guide RNAs". These guide RNAs are also referred to as siRNAs, or short interfering RNAs. Evidence indicates that siRNAs are produced when a nuclease complex, which recognizes the 3' ends of dsRNA, cleaves dsRNA (introduced directly or via a transgene or virus) ~22 nucleotides from the 3' end. Successive cleavage events, either by one complex or several complexes, degrade the RNA to 19-20 bp duplexes (siRNAs), each with 2-nucleotide 3' overhangs. RNase III-type endonucleases cleave dsRNA to produce dsRNA fragments with 2-nucleotide 3' tails, thus an RNase III-like activity appears to be involved in the RNAi mechanism. Because of the potency of RNAi in some organisms, it has been proposed that siRNAs are replicated by an RNA-dependent RNA polymerase (Hammond et al., 2001, Nature Rev Gen 2:110-119; Sharp, 2001, Genes Dev 15: 485-490).

In the effector step, the siRNA duplexes bind to a nuclease complex to form what is known as the RNA-induced silencing complex, or RISC. The nuclease complex responsible

for digestion of mRNA may be identical to the nuclease activity that processes input dsRNA to siRNAs, although its identity is currently unclear. In either case, the RISC targets the homologous transcript by base pairing interactions between one of the siRNA strands and the endogenous mRNA. It then cleaves the mRNA ~12 nucleotides from the 3' terminus of the siRNA (Hammond et al., 2001, *Nature Rev Gen* 2:110-119; Sharp, 2001, *Genes Dev* 15: 485-490).

Methods and procedures for successful use of RNAi technology in post-transcriptional gene silencing in plant systems has been described by Waterhouse et al. (Waterhouse et al., 1998, *Proc Natl Acad Sci U S A*, 95(23):13959-64). Methods specific to construction of the RNAi constructs of the invention can be found in Examples 2 and 6 as well as Figures 6 and 10. While the invention encompasses use of any plant glycogenin-like gene of the invention in the RNAi constructs, in a preferred embodiment, the strands of RNA that form the dsRNA are complimentary strands encoded by a coding region on the 3' end from nucleotide residues 1196-1662 of SEQ ID NO:2.

For all of the aforementioned suppression or antisense constructs, it is preferred that such nucleic acid constructs express specifically in organs where starch synthesis occurs (i.e. tubers, seeds, stems roots and leaves) and/or the plastids where starch synthesis occurs. Alternatively, it may be preferred to have the suppression or antisense constructs expressed constitutively. Thus, constitutive promoters, such as the nopaline, CaMV 35S promoter, may also be used to express the suppression constructs. A most preferred promoter for these suppression or antisense constructs is a rice actin promoter. Alternatively, a co-suppression construct promoter can be one that expresses with the same tissue and developmental specificity as the plant glycogenin-like gene.

In accordance with the present invention, desired plants with suppressed target gene expression may also be engineered by transforming a plant cell with a co-suppression construct. A co-suppression construct comprises a functional promoter operatively associated with a complete or partial plant glycogenin-like nucleic acid molecule. According to the present invention, it is preferred that the co-suppression construct encodes fully functional plant glycogenin-like gene mRNA or enzyme, although a construct encoding a incomplete plant glycogenin-like gene mRNA may also be useful in effecting co-suppression.

In accordance with the present invention, desired plants with suppressed target gene expression may also be engineered by transforming a plant cell with a construct that can effect site-directed mutagenesis of the plant glycogenin-like gene. For discussions of nucleic acid constructs for effecting site-directed mutagenesis of target genes in plants see, e.g., Mengiste et al., 1999, *Biol. Chem.* 380:749-758; Offringa et al., 1990, *EMBO J.* 9:3077-84; and Kanevskii et al., 1990, *Dokl. Akad. Nauk. SSSR* 312:1505-7. It is preferred that such constructs effect suppression of plant glycogenin-like genes by replacing the endogenous plant glycogenin-like gene nucleic acid molecule through homologous recombination with either an inactive or deleted plant glycogenin-like protein coding nucleic acid molecule.

In yet another embodiment, antisense technology can be used to inhibit plant glycogenin-like gene mRNA expression. Alternatively, the plant can be engineered, e.g., via targeted homologous recombination to inactive or "knock-out" expression of the plant's endogenous plant glycogenin-like protein. The plant can be engineered to express an antagonist that hybridizes to one or more regulatory elements of the gene to interfere with control of the gene, such as binding of transcription factors, or disrupting protein-protein interaction. The plant can also be engineered to express a co-suppression construct. The suppression technology may also be useful in down-regulating the native plant glycogenin-like gene of a plant where a foreign plant glycogenin-like gene has been introduced. To be effective in altering the activity of a plant glycogenin-like protein in a plant, it is preferred that the nucleic acid molecules are at least 50, preferably at least 100 and more preferably at least 150 nucleotides in length. In one aspect of the invention, the nucleic acid molecule expressed in the plant cell can comprise a nucleotide sequence of the invention which encodes a full length plant glycogenin-like protein and wherein the nucleic acid molecule has been transcribed only in the antisense direction.

In a particular embodiment of the invention, a plant is engineered to express a dsRNA homologous to a portion of the coding region of an endogenous PGSIP or a plant glycogenin-like gene transcribed in the antisense direction in order to alter the starch content of the plant. In a preferred embodiment, the starch content is 40%, 30%, 20%, 10%, 5% less than that of a non-engineered control plant(s). In another preferred embodiment, starch is absent from certain plant organs or tissues in comparison to a non-engineered control

plant(s). In one embodiment starch content is decreased or absent in the leaves of plants engineered using the antisense technology described herein when compared to the starch content in a non-engineered control plant(s). In other embodiments the starch content of tubers, or seeds is decreased or absent in plants engineered using the antisense technology described herein when compared to the starch content in a non-engineered control plant(s). Plant tissues in which starch content can be decreased using the methods of the invention include but are not limited to endosperm, leaf mesophyll, and root or stem cortex or pith.

In another aspect of the invention, the nucleic acid molecules of the invention are expressed in a plant cell engineered expressing a dsRNA homologous to a portion of the coding region of an endogenous PGSIP or using the antisense technology described herein and the starch content of the plant cell and plants derived from such a cells exhibit altered starch content. The altered starch content comprises an decrease in the ratio of amylose to amylopectin. In one embodiment of the invention, the ratio of amylose to amylopectin decreases by 10%, 20%, 30%, 40%, or 50% in comparison to a non-engineered control plant(s).

In a particular embodiment, the nucleic acid molecules of the invention are expressing a dsRNA homologous to a portion of the coding region of an endogenous PGSIP or using the antisense technology described herein, in conjunction with a developmental specific promoter directed towards later stages of development. In this particular embodiment, starch content in leaves of a plant can decrease, while starch content in other organs and tissues of a plant are altered in the same or different ways.

In another particular embodiment, the nucleic acid molecules of the invention are expressing a dsRNA homologous to a portion of the coding region of an endogenous PGSIP or using the antisense technology described herein in conjunction with a developmental specific promoter directed towards later stages of seed development, in cereals crops. In this embodiment, the ratio of small starch granules to large starch granules increases. An increased ratio of small to large starch granules results in greater accessibility of starch granules, which has certain industrial and commercial advantages related to extraction and processing of starch.

The progeny of the transgenic or genetically-engineered plants of the invention

containing the nucleic acids of the invention are also encompassed by the invention.

1.5 MODIFIED STARCH

The invention encompasses methods of altering starch synthesis in a plant and the resulting modified starch produced.

In the context of the present invention, "altering starch synthesis" means altering any aspect of starch production in the plant, from initiation by the starch primer to downstream aspects of starch production such as elongation, branching and storage, such that it differs from starch synthesis in the native plant. In the invention, this is achieved by altering the activity of the starch primer, which includes, but is not limited to, its function in initiating starch synthesis, its temporal and spatial distribution and specificity, and its interaction with downstream factors in the synthesis pathway. The effects of altering the activity of the starch primer may include, for example, increasing or decreasing the starch yield of the plant; increasing or decreasing the rate of starch production; altering temporal or spatial aspects of starch production in the plant; altering the initiation sites of starch synthesis; changing the optimum conditions for starch production; and altering the type of starch produced, for example in terms of the ratio of its different components. For example, the endosperm of mature wheat and barley grains contain two major classes of starch granules: large, early formed "A" granules and small, later formed "B" granules. Type A starch granules in wheat are about 20 μm diameter and type B around 5 μm in diameter (Tester, 1997, in : Starch Structure and Functionality, Frazier et al., eds., Royal Society of Chemistry, Cambridge, UK). Rice starch granules are typically less than 5 μm in diameter, while potato starch granules can be greater than 80 μm in diameter. The quality of starch in wheat and barley is greatly influenced by the ratio of A-granules to B-granules. Altering the activity of the starch primer will influence the number of granule initiation sites, which will be an important factor in determining the number and size of formed starch granules. The degree to which the starch priming activity of the plant is affected will depend at least upon the nature and of the nucleic acid molecule or antagonist introduced into the plant, and the amount present. By altering these variables, a person skilled in the art can regulate the degree to which starch synthesis is altered according to the desired end result.

The methods of the invention (i.e. engineering-a plant to express a construct comprising a plant glycogenin-like nucleic acid) can, in addition to altering the total quantity of starch, alter the fine structure of starch in several ways including but not limited to, altering the ratio of amylose to amylopectin, altering the length of amylose chains, altering the length of chains of amylopectin fractions of low molecular weight or high molecular weight fractions, or altering the ratio of low molecular weight or high molecular weight chains of amylopectin. The methods of the invention can also be utilized to alter the granule structure of starch, i.e. the ratio of large to small starch granules from a plant or a portion of a plant. The alteration in the structure of starch can in turn effect the functional characteristics of starch such as viscosity, elasticity, or rheological properties of the starch as measured using viscometric analysis. The modified starch can also be characterized by an alteration of more than one of the above- mentioned properties.

In an embodiment the length of amylose chains in starch extracted from a plant engineered express a construct comprising a plant glycogenin-like nucleic acid is decreased by at least 50, 100, 150, 200, 250, or 300 glucose units in length in comparison to amylose from non-modified starch from a plant of the same genetic background. In another embodiment, the length of amylose chains in starch is increased by at least 50, 100, 150, 200, 250, or 300 glucose units in length in comparison to amylose from non-modified starch from a plant of the same genetic background.

In an embodiment of the invention, the ratio of amylose to amylopectin decreases by 10%, 20%, 30%, 40%, or 50% in comparison to a non-engineered control plant(s).

In a preferred embodiment, the ratio of low molecular weight chains to high molecular weight chains of amylopectin is altered by 10%, 20%, 30%, 40%, or 50% in comparison to a non-engineered control plant(s).

In another preferred embodiment the average length of low molecular weight chains of amylopectin is altered by 5, 10, 15, 20, or 25 glucose units in length in comparison to a non-engineered control plant(s). In yet another preferred embodiment the average length of high molecular weight chains of amylopectin is altered by 10, 20, 30, 40, 50, 60, 70, or 80 glucose units in length in comparison to a non-engineered control plant(s).

According to one aspect of the invention, the ratio of small starch granules to large

granules is altered by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more in comparison to a non-engineered control plant(s).

In another aspect, the invention provides a complex comprising plant glycogenin-like proteins and plant polysaccharides. The inventors believe that members of the family of plant glycogenin-like proteins serve as primers for biosynthesis of a range of polysaccharides in plants, including but not limited to starch, hemicelluloses, and cellulose. The plant polysaccharides may be either homopolysaccharides comprising only a single type of monomeric unit or a heteropolysaccharides comprising two or more different kinds of monomeric units. Accordingly, it is contemplated that plant glycogenin-like proteins form complexes with such polysaccharides and its subunits. Glycosylated plant glycogenin-like proteins are encompassed in the invention. In the broadest sense, the invention encompasses a complex comprising a plant glycogenin-like protein and a number of monomeric units also referred to as subunits of the polysaccharides. Examples of monomeric units include but are not limited to glucose, xylose, mannose, galactose, ribose, and rhamnose, and may be a hexose, or a pentose, wherein the number ranges from a single to thousands of monomeric units, and wherein the linkages between the subunits may vary resulting in linear and/or branched structures. For example, starch and precursors of starch comprise of glucose subunits joined by either alpha 1, 4-glycosidic bonds or alpha 1, 6-glycosidic linkages; cellulose and precursors of cellulose comprise glucose subunits joined by beta 1, 4-glycosidic bonds. The number of monomeric units ranges from 1-3, 2-5, 4-10, 8-16, 15-30, 20-40, 30-60, 50-100, 75-200, 100-500, or 300-800 monomeric units. Alternatively, the number of monomeric units ranges from 1000-5000, 5000-10,000, or 10,000-15,000 monomeric units. Preferably, the polysaccharide or its precursor is attached to a hydroxyl group of a tyrosine residue of the plant glycogenin-like protein. Without being bound by any theory or any mechanism, during biosynthesis, additional subunits, either singly or as oligosaccharides are added to the complex such that the total number of subunits increase over a period of time.

In one embodiment, the invention encompasses complexes comprising plant glycogenin-like protein and starch. In a specific embodiment, the complexes of plant glycogenin-like protein and starch are purified. The starch molecule or its precursor including a single glucose subunit, can be attached to a hydroxyl group of a tyrosine residue

of the plant glycogenin-like protein. In various embodiments, in a population of complexes, the starch molecules that are complexed with the plant glycogenin-like proteins have different chain lengths and branching structures, for example, 1-3, 2-5, 4-10, 8-16, 15-30, 20-40, 30-60, 50-100, 75-200, 100-500, 200-700 glucose subunits. The polysaccharide complexed with the plant glycogenin-like proteins may consists of 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, or 190 glucose subunits in length. In preferred embodiments of the invention, the polysaccharide is amylopectin, amylose, or a combination of both.

The complexes of the invention can be used to identify sites of starch synthesis in stages of plant development. Briefly, the glycogenin-like protein can be labeled by means described herein and the complexes from tissues, cells, or organs can then be separated by size and compared among different stages of development.

The embodiments described in each section above apply to the other aspects of the invention, *mutatis mutandis*.

EXAMPLES

EXAMPLE 1: Identification of Plant Glycogenin-like Gene Homologues in *Arabidopsis*

Arabidopsis nucleic acid molecules showing similarities to yeast glycogenin genes were identified by sequence analysis. The sequence analysis programs used in the following examples are from the Wisconsin Package of computer programs (Deveraux et al., *Nucl. Acids Res.* 12: 387 (1984); available from Genetics Computer Group, Madison, WI). ESTs and genes were identified using the program BLAST (Basic Local Alignment Search Tool; Altschul, S.F. et al (1990) *J. Mol. Biol.* 215:403-410, see also www.ncbi.nlm.nih.gov/BLAST/).

The sequence comparison and identification program tblastx was used with the yeast glycogenin 1 (Glg1) gene (GenBank:U25546, Swiss_Prot (SP):P36143) to search against the *Arabidopsis* sequences collected in an in-house database comprising published plant

sequences. A number of hits to this gene were obtained. One of the hits was identified as EMBL:AC004260 version GI:2957150 which was annotated as "Sequencing in progress." Therefore, the region showing homology to the yeast Glg1 gene was extracted and a protein sequence was predicted using GENSCAN (a protein prediction program, Burge, C. and Karlin, S. (1997), J.Mol.Biol., <http://genes.mit.edu/GENSCANinfo.html>). A blastp analysis using this protein showed strong homology to the glycogenin genes from *C.elegans* (8e-22), human (2e-19) and yeast (8e-06). A search in the database at NCBI at a later date showed that this gene is listed as T14N5.1 with the accession number EMBL:AC004260 (SPTREMBL:O80649) and annotated as "Unknown protein". The protein sequence is set forth in SEQ ID NO: 6.

The in-house database described above was also searched with the yeast Glg2 gene (GB:U25436, SP:P47011) and the sequence identified above (accession EMBL:AC004260) using the program tblastn and tblastx. A number of further hits were identified. Out of the list of best hits, accession no. EMBL:AB026654, gene_id:MVE11.2 (SPTREMBL:Q9LSB1), showed strong homology to the glycogenin genes from *C.elegans* (1e-21), GYG2 human (3e-21) and yeast (5e-06). The genomic sequence representing this gene was extracted and is shown in SEQ ID NO: 1. Further analysis by the organelle prediction programs PREDOTAR and/or TargetP (Emanuelsson *et al.*, J. Mol. Biol. 300: 1005-1016 (2000)) showed that the protein comprises a transit peptide as shown in Table 1 below.

Table 1. TargetP V1.0 Prediction Results.

Number of input sequences: 1

Cleavage site predictions included.

Using PLANT networks.

| Name | Length | cTP | mTP | SP | Other | Loc. | RC | TPlen |
|-----------|--------|-------|-------|-------|-------|------|----|-------|
| AT3g18660 | 659 | 0.792 | 0.181 | 0.004 | 0.172 | C | 2 | 65 |
| cDNA | | | | | | | | |

Performing blastp analysis using this protein against yeast sequences in an in-house database clearly showed sequence similarities to the yeast Glg1 and Glg2 gene. were and a

CD-ROM containing the full genome sequence of *Arabidopsis* was made available. A search of the *Arabidopsis* genome sequencing project database published (Nature 408: 791, (2000)) showed that EMBL:AB026654 corresponded to the sequence having accession no. AT3g18660. However AT3g18660 is reported to encode a protein of 575 amino acids whereas our analysis shows that this gene actually encodes a protein of 659 amino acids. A blastp analysis against the in-house database showed strong hits to five genes, EMBL:AC004260, AC000106, AC069144, AL035678 and AL035678 (corresponding to MIPS:at1g77130, at1g08990, at1g54940, at4g33330 and at4g33340). The sequences of these five genes are shown in SEQ ID NOS: 6, 8, 10, 12 and 14. The different accession numbers of these genes and their description in various databases are presented in Table 2.

Table 2:

Accession numbers of the genes in various databases:

| MIPS | SPTREMBL | EMBL | GENE | Size |
|-----------|----------|----------|-----------|---------------------|
| AT3g18660 | Q9LSB1 | AB026654 | MVE11.2 | 659 ^a aa |
| at1g77130 | O80649 | AC004260 | T14N5.1 | 1201aa |
| at1g08990 | O 04031 | AC000106 | F7g19.14 | 546 ^b aa |
| at1g54940 | Q 9FZ37 | AC069144 | F14C21.47 | 557aa |
| at4g33330 | Q9SZB0 | AL035678 | F17M5.90 | 333aa |
| at4g33340 | Q9SZB1 | AL035678 | F17M5.100 | 277aa |

Note: ^a = The AT3g18660 gene sequence in the MATDB (MIPS) database is reported to encode a 575 aa protein. The analysis performed by the inventors indicates that (exon 2) of the AT3g18660 gene is missing in the MATDB (MIPS) database sequence and present in sequences of the AT3g18660 gene found in other databases.

^b = The at1g08990 gene accession in the MATDB (MIPS) database is reported to encode a protein of 550 aa in MATDB (MIPS). The at1g08990 gene accession in other databases is 546aa in length.

Table 3: Comparison of AT3g18660 with other glycogenin-like genes from *Arabidopsis*:

| | % identity nucleotide | % identity protein |
|-----------------------|-----------------------|--------------------|
| AT3g18660 X at1g77130 | 68 | 65 |
| AT3g18660 X at1g08990 | 61 | 50 |
| AT3g18660 X at1g54940 | 61 | 49 |
| AT3g18660 X at4g33330 | 60 | 58 |
| AT3g18660 X at4g33340 | 60 | 46 |

Table 2 shows the percentage identity between AT3g18660 and other glycogenin genes from *Arabidopsis* using the programme BESTFIT of the GCG package. In each case, the full length nucleotide and peptide was compared to the AT3g18660 gene.

These levels of identity are consistent with the genes encoding proteins with the same function. For example, the two yeast glycogenin genes are about 50% identical to one another at the protein level and are both known to be involved in the same pathway; both are essential for the production of glycogen and one can complement for the function of the other.

It is interesting that the carboxyl terminal region of the protein encoded by at1g77130 shows homology to a starch synthase (dull1) from maize. In yeast, glycogenin and glycogen synthase physically interact. This finding may be the first indication that a similar scenario exists in plants. The at1g77130 gene appears to be a duplication of the AT3g18660 sequence, and the small region of homology with dull1 may indicate that during the course of evolution this gene has become physically close to dull1. Recently published work (Yanai et al ., 2001, Proc. Natl. Acad. Sci. USA 98(14): 7940-7945) suggests that a functional association between two genes can be derived from the existence of a fusion of the two as one continuous sequence in another genome. In yeast, it has been shown by experimentation that glycogenin and glycogen synthase physically interact and are associated together in an enzymatic complex to allow glycogen biosynthesis. The inventors believe that PGSIP interacts with soluble starch synthases at the start of the starch biosynthesis process. This could be the first

step in the formation of a biosynthetic starch enzymes complex where PGSIP acts as a template, starch synthases extend the chain followed by branching by starch branching enzymes and other starch synthesis enzymes. It is likely that biosynthesis starch enzymes become associated with the very first complex formed in the process of the synthesis of a starch polymer.

The sequences of the six genes listed in Table 2 were compared by BLAST against the *Arabidopsis* sequences in an in-house database and a further hit was obtained. The identified sequence corresponding to SPTREMBL: Q8W4AZ, EMBL: AY062695 encodes a protein of 618 amino acids that showed strong homology to the glycogenin genes (4e-26). Further analysis of the sequence indicated that the protein represents the C terminal domain of the At1g77130 gene (O80649, T14N5.1) and is also annotated as At1g77130, T14N5.1 which encodes an unknown protein. This sequence is set forth in SEQ ID NO: 23.

EXAMPLE 2: Isolation of cDNA Encoding *A. thaliana* Glycogenin Homologue

Primers were designed to clone a full length cDNA representing the accession number AB026654, gene_id:MVE11.2 (at3g18660 (MIPS)) from an *Arabidopsis thaliana* cDNA pool. Sequencing the full length clone indicated that the gene encoded a protein of 659 amino-acids and consists of five exons. The cDNA sequence designated as SEQ ID NO: 2.

Arabidopsis thaliana was grown in growth cabinets with a 16 hours light and 8 hours dark period at a temperature of 22°C during the day and 17°C during the night. A mixed cDNA sample was made with total RNA from 10 different tissues mixed together in equal amounts: root, dividing cell culture, young leaf, mature leaf, stem, seedling, seed, flower buds + flowers, drought 6 days- and drought 10 days-subjected plants.

The primer used to make the first strand cDNA using Superscript II was from the original paper on PCR amplification by (Frohman *et al.* (1988) Proc. Natl. Acad. Sci. USA, 85:8998):

5 'GACTCGAGTCGACATCGATTTTTTTTTTTTT 3'.

1 µl of this cDNA was used to amplify the cDNA clone representing the accession number GTD:S:1870408 (gene id:MVE1 1.2) utilizing the primers G1gfl and G1g int1 and C1aF and

Glgstop2.

G1gfl primer: 5'-GACCATGGCAAACTCTCCCGC-3'

G1g int1 primer: 5'-GCAGCATACTTTCCAATTAC-3'

C1aF primer: 5'-GCAAGTTCCGGCTATGGCAGC-3'

Glgstop2 primer: 5'-GCGTCACAAGTTATGGCCGGG-3'

PCR conditions:

Five 50 μ l reaction was set up as follows:

| Composition | PCR Programme | | |
|---------------------------|---------------|------|--------------------|
| Water..... | 35.5 μ l | 95°C | 2 min (hot start) |
| 10xbuffer..... | 5 μ l | 95°C | 3 min |
| 4mMdNTPs..... | 2.5 μ l | 55°C | 30 sec |
| Pfu Turbo polymerase..... | 1 μ l | 72°C | 2 min:30 sec |
| 4mM primers..... | 5 μ l | 72°C | 10 min (extension) |
| cDNA..... | 1 μ l | | |

Two products were obtained. These were cloned in pBluescript vector (SK-) (Stratagene) and a full length clone was obtained. The map of this plasmid is shown in Figure 1.

EXAMPLE 3: Functional Analysis of The *Arabidopsis* cDNA

Yeast contains two glycogenin genes Glg1 (YKROS8w) and Glg2 (YJL137c). Double mutants in the above genes do not make any glycogen (Cheng *et al* (1995) Mol. and Cell Biology 15(12):6632-6640). Mutant yeast strains from the EUROSCARF (European

Saccharomyces Cerevisiae ARchives For Functional Analysis collection were obtained from SRD GmbH, D61440, Germany along with the wild type. Single mutants in the *Glg1* and *Glg2* genes were obtained in addition to the double mutant. Additionally a plasmid containing the entire *Glg2* ORF including the promoter was also obtained. This plasmid was used as a positive control to establish a complementation assay. The description of the strains are:

Wild type

| ORF | Accession no. | Strain | Genotype |
|-----|---------------|--------|--|
| | Y00000 | BY4741 | MAT α ; his3 Δ 1; leu2 Δ 0; met15 Δ 0; ura3 Δ 0 |

Single mutants:

| ORF | Accession no. | Strain | Genotype |
|---------|---------------|-------------|---|
| YKR058W | Y15129 | G1G1 mutant | BY4742; Mat alpha; his3 Δ 1; leu2 Δ 0; ura3 Δ 0; YKR058w::kanMX4 |
| YJL137c | Y17003 | g1g2 mutant | BY4742; Mat α ; his3 Δ 1; leu2 Δ 0; ura3 Δ 0; YJL137c::kanMX4 |

Double mutants:

| Mutant Strains | Genotype |
|-----------------------------|---|
| 1. <i>g1g1/glg2</i> deleted | BY4742; Mat alpha; his3 Δ 1; leu2 Δ 0; ura3 Δ 0; YKR058w::kanMX4; YJL137c::kanMX4 |

| | |
|-----------------------------|---|
| 2. <i>g1g1/glg2</i> deleted | BY4742; Mat a; <i>his3</i> Δ1; <i>leu2</i> Δ0; <i>ura3</i> Δ0; YKR058w::kanMX4; YJL137c::kanMX4 |
|-----------------------------|---|

Plasmid

| Plasmid name | Gene | Marker |
|----------------------|--------------------------|--------|
| PYCG_YJL137c(pRS416) | <i>G1g2</i> ORF+promoter | URA3 |

Glycogen defect assay

First, it was established that the wild type and the double mutants were indeed different. For this experiment, freshly grown wild type, and the double mutants were picked up from YPD plates and the cells were suspended in 100 μl of water in an eppendorf tube. To this tube approximately 100 μl of glass beads (Sigma) and 10-20 μl of undiluted Lugol solution (Sigma) was added. The cells were vortexed briefly, spun down for few seconds and assayed for color development. The wild type cells stained brown whereas the double mutants did not stain and appeared yellow.

Complementation assay

Double mutants were transformed with the plasmid pRS416 and the transformants were selected on CSM/Ura- plate (Uracil drop out plate). As a negative control, double mutants were transformed without the plasmid. Many colonies were obtained in the positive plate but no colonies were obtained from the negative control indicating that the transformation had worked. The transformed double mutants were grown overnight in CSM/Ura- liquid media along with wild type and single mutants. Next day OD₆₀₀ was checked to ensure equal amounts of cells in each of the tubes. Approximately equal amounts of cells were taken in an eppendorf tube and to this equal amounts of glass bead were added followed by 10-20 μl of undiluted Lugol solution (Sigma). The cells were vortexed briefly and centrifuged for few seconds and assayed for colour development. Complementation was observed in the double mutants as they appeared blue similar to the single *g1g1* and *glg2* mutants.

Optimisation of the assay to distinguish wildtype and mutant strains

A small amount of the wildtype (WT) and glycogenin double mutant (Mut) yeast strains were picked up from a well-grown plate, resuspended in 1ml of water, and vortexed briefly. The cells were diluted further in 1ml of water and 50ul of the diluted cells were plated on YPD plates. The plate was incubated at 30°C for two days and afterwards the plates were exposed to iodine vapour by inverting the plates on top of a 500ml glass beaker containing iodine chips (Sigma) placed on a low heater under a fume cupboard briefly for 2-3 minutes. Afterwards the plates were left open in the fume cupboard briefly for 1 minute and the colour development was monitored. The WT cells stained brown and the double mutants (Mut) stained pale yellow.

Cloning PGSIP cDNA in into the pYES2 vector for complementation studies

Two constructs were made to do the experiment, one contained the full length PGSIP cDNA including the transit peptide (TP) and another in which the transit peptide was removed (No transit peptide : NTP), these were cloned into pYes2 vector (Invitrogen). Primers were designed to amplify the full length PGSIP cDNA with the transit peptide (primers TPF and TPR) and without the transit peptide (primers NTPF and NTPR) so that these could be cloned into the pYes2 vector. A BamHI restriction enzyme site was incorporated into the forward primers (TPF and NTPR) and a XhoI restriction enzyme site was incorporated into the reverse primers (TPR and NTPR). The NTP forward primer (NTPF) was designed in such a manner so that it annealed at nucleotide position 190 of the full length PGSIP sequence and an ATG initiation codon was inserted after the BamHI site to ensure that translation into protein could occur. This resulted in a cDNA sequence lacking the first 63 amino acids of the PGSIP cDNA sequence which represents the transit peptide as predicted by the Target P program (Emanuelsson et al, J. Mol. Biol. 300:1005-1016 (2000). The primer sequences were as follows:

TPF 5'-GGATCCGACCATGGCAAACTCTCCCGC-3'

TPR 5'-CTCGAGGCGTCACAAGTTATGGCCGGG- 3'

NTPF 5'- GGATCCATGTGTTGTTACCAAG-3'

NTPR 5'-CTCGAGGCGTCACAAGTTATGGCCGGG-3'

A 50 μ l PCR reaction was set up with Pfu polymerase (Stratagene) as follows: a coocktail solution was made with 35.5 μ l water, 5 μ l 10X PCR buffer+, 2.5 μ l solution (20mM MgCl and 4mM dNTPs), 1 μ l Pfu polymerase, 5 μ l 4mM primers (TP/NTP), and 1 μ l cDNA (1/100dil). The PCR thermocycler program consisted of a 95°C 3min (hot start), followed by 30 cycles of 95°C for 30sec, 50°C for 30sec, and 72°C for 3min. The final step in the program held the temperature at 24°C.

The amplified fragment was run out on an agarose gel, cut out and purified using the 'Geneclean kit' according to the manufacturers instructions (Bio101). The purified cDNA fragments were ligated into pBluescript vector (Stratagene) cut with EcoRV restriction enzyme. Positive clones were identified and these were sequenced. Clones with the correct sequences were then cut with the restriction enzymes BamHI and XhoI and ligated in pYes2 vector cut with the restriction enzymes BamHI and XhoI. Positive clones were identified and these were named, pTPYYes (Figure 2) and pNTPYYes (Figure 3). In these plasmids, the cDNA was under the control of the yeast Gal 1 promoter that is both glucose repressible and galactose inducible.

*Complementation analysis with the *Arabidopsis* glycogenin gene*

Yeast strains were transformed with the above plasmids following the method of Finley and Brent, 1995, (<http://cmmg.biosci.wayne.edu/finlab/YTHprotocols.htm> and links there in) in combination with the Clontech yeast transformation kit. From a freshly grown plate a 5ml culture of yeast strain (WT and Mut) was inoculated in YPD medium (Clontech) overnight with shaking at 30°C. Next day, 3ml freshly grown cells were inoculated into 150ml YPD medium, (OD600=0.2) and grown shaking at 30°C for 3-4 hours (OD600=0.7). 100ml cells were then transferred to two 50ml orange cap tubes and centrifuged at room temperature at 2000rpm for 3 minutes. The supernatant was discarded completely. The cells

were washed by resuspending them in 2.5ml of sterile water followed by centrifugation as before. The supernatant was discarded and the cells were resuspended by adding 625ul of Lithium Acetate (LiAc)/TE (10mM Tris HCl pH 7.5, 1mM EDTA, 100mM LiAc; made from a filter-sterile stock of 1M LiAc, pH 7.5) in each tube. The cells were centrifuged as before and the supernatant was discarded. The cells were resuspended in 250ml of LiAc/TE then pooled into a single eppendorf tube giving 500ml of competent yeast cells. In an eppendorf tube the following was prepared, 6ml Herring Testis DNA (Clontech, 10mg/ml, boiled earlier for 10 minutes and quenched on ice), 8ml DNA [pYes2 empty plasmid, TPYes and NTP Yes DNA (~2ug)] and 6ml of water making a total volume of 20ml. In another tube 100ml of competent yeast cells were added to which the 20ml mixture made above, plus 11ml DMSO and 600ul of 40% PEG 4000 in LiAc/TE (made from stocks of 1M LiAc pH 7.5, filter sterile 50% PEG 4000 in water, 1M Tris HCl pH 7.5 and 0.5M EDTA) was added. The tubes were inverted three to four times gently and incubated at 30°C for 30 minutes. The tubes were inverted again gently and heat shocked at 42°C for 20minutes after which 50-100ml was directly plated on CSM/Ura-/glucose plates. The plates were incubated for two to three days at 30°C. Additionally, as a negative control, WT and Mut yeast strains were transformed with the empty pYes2 plasmid. As a positive control the Mut strains were transformed with the yeast GLG2 gene (plasmid pRS416) purchased from EUROSCARF. The transformed cells were selected on CSM/Ura- glucose drop out plates. After two days the cells were picked individually into patches and streaked onto glucose and galactose plates. In the end, we had the following plates.(Table 4)

Table 4

| Name | Glucose | Galactose |
|----------------------|---------|-----------|
| 1. WT:pYes2 control | | Yes |
| 2. Mut:pYes2 control | Yes | Yes |
| 3. WT:NTP | Yes | Yes |
| 4. Mut:NTP | Yes | Yes |
| 5. WT:TP | Yes | Yes |

| | | |
|---------------------------------------|-----|-----|
| 6. Mut:TP | Yes | Yes |
| 7. Mut:yeast GLG2 gene +ve control | Yes | Yes |

Yeast strains used for the complementation experiment (Table 5)

Table 5

| Name |
|----------------------|
| 1. WT:pYes2 control |
| 2. Mut:pYes2 control |
| 3. Mut:TP |
| 4. Mut:NTP |
| 5. Mut:yeastGLG2 |

The plates listed in Table 4 and Table 5 were grown for two days at 30°C as described above. The cells were diluted and plated on to both CSM/Ura- glucose and CSM/Ura-galactose plates. After two days of growth at 30°C the cells were exposed to iodine vapour as described above and photographs were taken. From the photographs, it was confirmed that the assay worked as the Mut strains containing the yeast GLG2 gene (no.7 from the table 4) stained brown both in the glucose and galactose plates. The WT strain (no.1 from the table 4) stained brown whereas the Mut strains (no. 2 from the table 4) containing the empty plasmid stained yellow. The cells containing the NTP plasmid (no. 4 from the table 4) stained yellow in glucose plate but it stained brown in galactose plates but the brown colour is not as intense as observed in Mut strains containing the yeast GLG2 gene indicating that the complementation is partial. This data indicates that the PGSIP cDNA is a functional orthologue of the yeast glycogenin gene and plays a role in starch biosynthesis especially in plants and particularly in *Arabidopsis*. The cells containing the TP plasmid (no. 3 from the table 4) stains yellow in glucose and galactose plates indicating that complementation was not achieved with this plasmid. In general, validating the function of plant genes by yeast complementation has been reported (Alderson et al, Proc. Natl. Acad. Sci. USA, 88:8602-

8605 (1991), Vogel et al., Plant J, 13 (5):673-683, 1998, Blazquez, et al., Plant J, 13 (5):685-689, 1998.

EXAMPLE 4: cDNA Isolation from Maize Endosperm

Maize EST identification

ESTs encoding corn glycogenin gene were identified using the program BLAST (Basic Local Alignment Search Tool; Altschul, S.F. *et al* (1990) J. Mol. Biol. 215:403-410, see also www.ncbi.nlm.nih.gov/BLAST/). A database search using the *Arabidopsis* gene AT3g18660 and atlg77130 against the maize database at NCBI identified accession no. GB: BF729544 and GB: BG837930 which showed significant similarity to the *Arabidopsis* glycogenin genes. The sequence of the two ESTs is shown in SEQ ID NO: 4, and SEQ ID NO: 5 respectively. A blastx analysis of the two ESTs against SPTREMBL database showed that EST BF729544 picked up the first hit to the AT3g18660 gene whereas EST BG837930 showed first hit to the atlg77130 gene. Protein alignments of these ESTs indicated that both ESTs were partial and they showed 85-86% identity to the above two *Arabidopsis* genes. Moreover, for EST BF729544 the identity was confined to the central portion of the AT3g18669 protein starting at amino-acid position 245 and ending at position 427, whereas for EST BG837930 the identity started at amino-acid position 391 and extending until position 632. A bestfit analysis between the two nucleotide sequences of the ESTs and the AT3g18660 gene showed that the two ESTs have 68-69% identity. A bestfit analysis between the two EST DNA sequences showed that there was a high degree of homology between the two ESTs. From the above analysis, it appears that EST BF729544 is the homolog of the *Arabidopsis* AT3g18660 gene, whereas EST BG837930 is a homolog of the *Arabidopsis* AT1g77130.

A database search using the *Arabidopsis* genes AT3g18660 and atlg77130, against the maize database in-house identified four additional sequences which showed significant similarity to the *Arabidopsis* glycogenin genes. The four nucleotide sequences called Maize SEQ 1, Maize SEQ 2, Maize SEQ 3 and Maize SEQ 4 are shown in SEQ ID NOs: 27, 29, 31 and 33 and the deduced amino acid sequences for these nucleotide sequences are shown in SEQ ID NOs: 28, 30, 32 and 34.

Culture conditions

Maize was grown in the greenhouse with a 16 hour daylight and 8 hour night period with a temperature of 24°C during the day and 18°C during the night. Seeds were harvested at different stages between 3 and 35 days after pollination (DAP). Young and medium leaves were also harvested.

*Establishment of copy number and identification of glycogenin homolog in maize, wheat and *Arabidopsis**

Genomic DNA was isolated from *Arabidopsis*, wheat and maize leaves according to the method of Davies *et al.*, ((1994) Methods in Molecular Biology vol. 28: Protocols for nucleic acid analysis by non-radioactive probes, Isaac P.G. (ad) pp 9-15 Humana press, Totowa, NJ USA). DNA was digested with restriction enzyme, EcoRI, XhoI and EcoRV and the digested DNA was run overnight at 20V in 1% agarose gels. The DNA was then transferred to a nylon membrane by vacuum blotting and two identical southern blots were prepared and each one was probed first at a high stringency and later at low stringency conditions. One blot was probed with a digioxygenin labelled AT3g18660 cDNA probe encoding the N-terminus of the gene (a 1.8kb NcoI-AvaI fragment) and filter 2 was probed with AT3g18660 cDNA probe (PGSIP) encoding the C-terminus of the gene (a 700bp C1a K fragment), Figure 5C. Hybridisation was done at 65°C and the blots were first washed with 2 x 5 minutes with 2 x SSC, 0.1 x SDS and later with 0.1 x SSC and 0.1 x SDS at 65°C (high stringency washes). Strong single bands of the expected sizes (5.9kb in the Xho1 cut DNA, 4.6kb in the EcoR1 cut DNA and 5.1kb in the EcoRV cut DNA) were observed only in the lanes containing *Arabidopsis* DNA. No band was observed in the lanes containing maize and wheat DNA, as shown in Fig. 4B. Later the blots were stripped and these were re-probed at 55°C and washed at 60°C for 2 x 15 minutes with 2 x SSC, 0.5 x SDS (low stringency washes). Three bands were observed in the lane containing XhoI digested *Arabidopsis* DNA, two- three bands were observed in the lanes containing maize and wheat DNA, as shown in Fig. 5A and 5B. From the genomic sequence of the AT3g18660 gene it was known that it

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spanned two Xho I, EcoR1 and EcoRV sites. This demonstrated that PGSIP exists as a gene family comprising of about 2-3 genes in *Arabidopsis*, maize and wheat.

RNA extraction and first strand cDNA synthesis

Total RNA was extracted from the tissues described above using the method of Napoli *et al* (1990), Plant Cell, 2, 279-289 and in some cases using Qiagen RNA extraction kit following manufacturer's protocol. First strand cDNA was made using SuperscriptII reverse transcriptase (GIBCO-BRL) and oligo dT primer as described in (Frohman *et al*, (1988), Proc. Natl. Acad. Sci. USA, 85:8998):

5' GACTCGAGTCGACATCGATTTTTTTTTTTTT 3'.

This cDNA pool was used to amplify a maize cDNA homolog to the *Arabidopsis* glycogenin gene (AT3g18660 and at1g77130) utilising the sequence information from the ESTs, GB:BF729544 and GB: BG837930 described above.

EST BF729544 and BG837930 overlapped and these were combined to deduce a single maize PGSIP sequence. Primers were designed to amplify a maize cDNA clone corresponding to this sequence. Primer sequences were as follows.

[GlgmaF] 5'-GGCAATAGAGGAATTCATGTGC-3'

[GlgmaR] 5'-CGTGCAGAACTCGGACCACAG-3'

Construction of a Maize cDNA library

Total RNA was extracted from the various tissues described above (leaves and seeds ranging from 3-35 DAP). The RNA obtained was mixed in equal amounts. This RNA mixture was then used to make a maize cDNA library using SMART cDNA library construction kit (Clontech) following manufacturer's instruction.

Cloning of Maize cDNA

1ul of this first strand cDNA obtained above was used to amplify the cDNA clone represented by the ESTs by PCR using the primers GlgmaF and GlgmaR, the PCR product obtained was cloned into EcoRV cut pBlueScript (SK-) and positive clones were identified. These positive clones were sequenced to confirm that the product obtained indeed represented the sequence in the EST accession number, BF729544. This product was then used to screen the cDNA library and a full length clone was obtained. Similarly a cDNA clone represented by the EST accession no. BG837930 was also cloned.

The PCR conditions were the same as described before for cloning the *Arabidopsis* gene (AT3g18660) of SEQ ID NO: 2.

EXAMPLE 5: cDNA Isolation From Wheat Endosperm

A database search using the *Arabidopsis* genes AT3g18660 and at1g77130, against the wheat in-house database identified one sequence, which showed significant similarity to the *Arabidopsis* PGS1P genes (e-137). The sequence called Wheat SEQ1 is shown in SEQ ID NO: 20.

Culture conditions

Wheat variety NB1 (described in patent WO 00/63398) was grown in the glass house with a 16 hour daylight and 8 hour night period with 22°C during the day and 15°C during the night. Seeds were harvested at different stages between 5 and 20 days after pollination (DAP). Young and medium leaves were also harvested.

RNA extraction and first strand cDNA synthesis

Total RNA was extracted from the above tissues using the method of Napoli et al (1990) and in some cases using Qiagen RNA extraction kit following manufacturer's protocol. First strand cDNA was made using SuperscriptII reverse transcriptase (GIBCO-BRL) and oligo dT primer as described in (Frohman et al, (1988), Proc. Natl. Acad. Sci. USA, 85:8998. This cDNA pool was used to amplify a wheat cDNA homolog to the

Arabidopsis glycogenin gene (AT3g18660 and at1g77130) utilising the sequence information from the maize ESTs, NCBI accession no. BF729544 and BG837930 described above.

Wheat cDNA library making

Total RNA was extracted from the various tissues described above (leaves and seeds ranging from 7-30 days post anthesis (DPA). The RNA obtained was mixed in equal amounts. This RNA mixture was then used to make a wheat cDNA library using SMART cDNA library construction kit (Clontech). Additionally a genomic library from *Triticum tauschii*, var strangulata, accession number CPI 110799, described in (Rahman et al., 1997, Genome, 40:465-474) was also used in this study. The cDNA library from Wheat cv Wyuna described in (Li et al., 1999, Theor. Appl. Gen. 98:226-233) was also used in this study.

Cloning of wheat cDNA

Because a strong band was observed on southern blots probed with the *Arabidopsis* gene (AT3g18660), it was assumed that there is significant degree of homology between the *Arabidopsis*, maize and wheat DNA sequences. A comparison of the *Arabidopsis* and the maize EST sequences also suggested that this was the case. A wheat cDNA library was screened with probes made from the maize and the *Arabidopsis* glycogenin gene. A full length clone was obtained by restriction mapping and analysing the sequence of a number of positive clones.

PCR conditions

The PCR conditions were the same as described before for cloning the *Arabidopsis* gene (AT3g18660).

EXAMPLE 6: Agrobacterium Constructs

Construct making

The pSB111 Sulugi described in patent publication WO 00/63398 was used. Six different constructs were made, one each for maize, wheat and *Arabidopsis* in sense orientation and one each for maize, wheat and *Arabidopsis* in antisense orientation for

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constitutive expression. Another six set of constructs, were also made using seed specific promoters.

Two constructs were made, one for overexpression and another for downregulation of the Atglycogenin gene. For overexpression, the Atglycogenin gene was excised out from the plasmid (At3g18660 (PGSIP), Figure 1) with SalI-EcoRI digest and ligated in SalI-EcoRI cut pJIT65 resulting in plasmid pCL68. This plasmid was then digested with EcoRI-XbaI and the fragment was ligated into SalI-SmaI cut Nos-NptII SCV resulting in plasmid pCL68 SCV. In this plasmid the Atglycogenin is under 2x 35S promoter for constitutive expression.

For RNAi construct, first a fragment representing the 3' end of the Atglycogenin gene was amplified by PCR using ClaF and Glgstop2 primer (see example 2) and was cloned into pBluescript. The resulting construct was designated pMC167. Clones in both orientation were obtained and the clone with the fragment in reverse orientation was called pMC167inv. pMC167inv was cut with EcoRV-SmaI and ligated back resulting in plasmid pMC167del. pMC167del was cut with HindIII-BamHI and ligated into HindIII-BamHI cut pT7blue2 resulting in plasmid "GlycoinpT7Blue2" (pCL66). Another plasmid (called GlycogeninIRstep1, pCL67) was created by cutting pMC167inv with XbaI-EcoRV and ligating this fragment into XbaI-EcoRV cut pWP446A containing the AtSac25 intron1. Finally, plasmid "GlycoinpT7Blue2", pCL66 was cut with BamHI-SstI and the fragment ligated into BamHI-SstI cut "GlycogeninIRstep1", pCL67 resulting in plasmid pCL69. pCL69 was cut with EcoRI-XbaI and the fragment was ligated in SCV Nos-NptII at the SmaI-SalI site resulting in plasmid pCL76 SCV. In this plasmid the At glycogenin (PGSIP) RNAi is under 2x35S promoter for constitutive expression.

Figure 6 summarises the whole process and the maps of these plasmids are shown in Figures 9 and 10. The plasmids were transformed into the GV3101 *Agrobacterium* strain and the *Arabidopsis* plants were transformed.

EXAMPLE 7: Transformation of Wheat

Wheat plants transformed with the constructs of Example 6 were produced by the seed inoculation method described in patent publication WO 00/63398. *Solanum tuberosum* c.v. Prairie was transformed with pCL68 SCV and pCL76 SCV using the method of leaf disk

cocultivation essentially as described by Horsch *et al.* (Science 227: 1229-1231, 1985). The youngest two fully-expanded leaves from a 5-6 week old soil grown potato plant were excised and surface sterilised by immersing the leaves in 8% 'Domestos' for 10 minutes. The leaves were then rinsed four times in sterile distilled water. Discs were cut from along the lateral vein of the leaves using a No. 6 cork borer. The discs were placed in a suspension of *Agrobacterium tumefaciens* strain LBA4404 containing one of the two plasmids listed above for approximately 2 minutes. The leaf discs were removed from the suspension, blotted dry and placed on petri dishes (10 leaf discs/plate) containing callusing medium (Murashige and Skoog agar containing 2.5µg/ml BAP, 1 µg/ml dimethylaminopurine, 3% (w/v) glucose). After 2 days the discs were transferred onto callusing medium containing 500µg/ml Claforan and 50µg/ml Kanamycin. After a further 7 days the discs were transferred (5 leaf discs/plate) to shoot regeneration medium consisting of Murashige and Skoog agar containing 2.5µg/ml BAP, 10 µg/ml GA3, 500µg/ml Claforan, 50µg/ml Kanamycin and 3% (w/v) glucose. The discs were transferred to fresh shoot regeneration media every 14 days until shoots appeared. The callus and shoots were excised and placed in liquid Murashige and Skoog medium containing 500µg/ml Claforan and 3% (w/v) glucose. Rooted plants were weaned into soil and grown up under greenhouse conditions to provide tuber material for analysis.

Alternatively, microtubers were produced by taking nodal pieces of tissue culture grown plants onto Murashige and Skoog agar containing 2.5µg/ml Kanamycin and 6% (w/v) sucrose. These were placed in the dark at 19° C for 4-6 weeks when microtubers were produced in the leaf axils.

EXAMPLE 8: Transformation of Maize

Maize plants transformed with the constructs of Example 6 were produced by the seed inoculation method described in patent publication WO 00/63398.

EXAMPLE 9: Transformation of Potato

Transgenic potato plants expressing the *Arabidopsis* plant glycogenin-like gene in sense and antisense orientation were produced.

EXAMPLE 10: Characterisation of the Transgenic Lines

Transgenic plants were analysed by the following methods

For sense constructs, 20 T1 lines were analysed; for antisense constructs, 50 T1 lines were analysed. Plants transformed with sense and antisense sequences of the invention were observed to have altered starch synthesizing ability which was linked to the expression of the transgene.

For the maize, wheat, and potato lines examined, several techniques of analysis were employed. PCR-positive line identification, northern- RNA expression, southern-copy number detection, western-protein expression, amylogenin activity, starch structure and quality, and phenotype all confirmed the successful transformation of the maize, wheat, and potato.

EXAMPLE 11: cDNA Isolation from Rice

The six genes listed in Table 2 were blasted against the rice sequences collected in an in-house database and one new hit was obtained. The accession corresponded to SPTREMBL:Q94HG3, EMBL:AC079633 (SEQ. ID NO: 25) which encodes a protein of 614 AA and shows strong homology to the PGSIP gene (e-129).

EXAMPLE 12: *Arabidopsis* Transformation.

Arabidopsis thaliana c.v. Columbia plants were transformed according to the method of Clough and Bent 1998 Plant J. 16(6):735-743 (1998) with slight modification. Plants were grown to a stage at which bolts were just emerging. Phytagar 0.1% was added to the seeds and these were vernalized overnight at 4°C. We used 10-15 seeds per 3x5 inch pots. Seed was added onto the soil with a pipette, about 4-5 seeds per ml was dispersed. Seeds were germinated as usual (ie under humidity pots were covered until first leaves appeared and then over a two day period the lid was cracked and then removed). Plants were grown for about 4 weeks in the greenhouse (long day condition) until bolts emerged. The first bolts were cut to encourage growth of multiple secondary bolts. Bolts containing many unopened flower buds were chosen for dipping.

Growing the Agrobacterium culture

Aliquots of the *Agrobacterium* strain GV3101 carrying the constructs pCL68 SCV and pCL68 76 SCV were grown first as a 5ml culture in YEP containing Gentamycin (15ug/ml) and Kanamycin 20ug/ml. Next day, 2ml freshly grown culture was added to 400ml YEP media (10g Yeast Extract, 10g peptone, 5g NaCl, pH 7.0) in a 2 litre flask. and the flask was incubated at 28°C incubator with shaking overnight. Next day OD 600 of the cells was measured and found to be 1.8. Cells were divided into 2X Oakridge bottles and harvested by centrifugation at 5000rpm for 10 min in a GSA rotor at room temperature. The pellet was resuspended in 3 volumes of infiltration media so that the final concentration of the culture was 0.6. Infiltration media was prepared by adding the following. ½ Murashige and Skoog Salts, 1x Gamborg's Vitamins and 0.44uM Benzylamino Purine (10ul per L of a 1mg/ml stock), pH was adjusted to 5.7 with NaOH. Then 0.02% Silwet (200ul per 1L) was added and mixed into the solution.

Arabidopsis transformation by Dipping

500 ml of resuspended *Agrobacterium* was poured into a tray and plants were inverted into Agrobacterium solution in batches of 10 for 15 minutes. After 15 minutes the plants were lifted and the excess solution drained, The plants were transferred on their sides to a fresh tray containing tissue paper to allow further soaking of the solution and then transferred to propagating trays. The plants were immediately covered with lids to maintain humidity. After two days the lid was removed and the plants allowed to grow normally. They were not watered for one week until the soil looked dry. After flowereing was complete and the siliques on the plants were dry, all the seeds from one pot were harvested. The seeds were completely dried by keeping harvested seed in an envelope for one week

EXAMPLE 13: Selection of transformed *Arabidopsis thaliana* seed. __

Seed produced from transformed *Arabidopsis thaliana* c.v. Columbia plants was weighed into 10 mg aliquots, equivalent to about 500 individual seed, and placed into a sterile 15 ml tube. The seed was surface sterilised by treating with 10 ml of Teepol bleach/ Tween 20 solution (500 ml of 50% (v/v) Teepol bleach containing 1 drop of Tween 20) for five minutes. The seeds were then washed four times with 10ml Tween 20 in sterile water (1 drop Tween 20 in 500ml sterile

water). The seeds were then suspended in 5 ml sterile water and 5ml warm 0.5% agar, mixed carefully and then half of the seeds were spread over one petri dish containing half strength Murashige and Skoog agar medium and the other half over a second dish containing half strength Murashige and Skoog agar medium plus 50 µg/ml kanamycin. The plates were sealed and incubated at 4°C for 48hours. The plates were then transferred to a growth room under low light (2000 lux). Seed on both types of plate germinated but on the plates containing kanamycin non-resistant plants bleached and died within 7 days. Figure 8 demonstrates this selection of kanamycin resistant seedlings. After 14 days the resistant plants were transferred from the selective medium onto MS medium for a further 10 days before being transferred into soil. The plants were grown on to produce leaf material for further analysis.

EXAMPLE 14: Analysis of *Arabidopsis thaliana* Plants Transformed with pCL68 SCV for the Presence of the PGSIP Construct

For the pCL68 SCV transformed lines a total of 31 kanamycin resistant plants were obtained from four of the original floral dips. These were tested for the presence of the construct by PCR.

Genomic DNA extraction

Leaf material was taken from regenerated *Arabidopsis thaliana* plants transformed with pCL68 SCV and genomic DNA isolated. One leaf was excised from a plant growing in soil and placed in a 1.5ml eppendorf tube. The tissue was homogenised using a micropestle and 400µl extraction buffer (200mM Tris HCL pH 8.0; 250mM NaCl; 25mM EDTA; 0.5% SDS) was added and ground again carefully to ensure thorough mixing. Samples were vortex mixed for approximately 5 seconds and then centrifuged at 10,000rpm for 5 minutes. A 350µl aliquot of the resulting supernatant was placed in a fresh eppendorf tube and 350µl chloroform was added. After mixing, the sample was allowed to stand for 5 minutes. This was then centrifuged at 10,000rpm for 5 minutes. A 300µl aliquot of the supernatant was removed into a fresh eppendorf tube. To this was added 300µl of propan-2-ol and mixed by inverting the eppendorf several times. The sample was allowed to stand for 10 minutes. The precipitated DNA was collected by centrifuging at 10,000rpm for 10 minutes. The supernatant was discarded and the pellet air dried.

The pellet of DNA was resuspended in 50 μ l of distilled water and was used as a template in PCR.

PCR detection of PGSIP

A pair of optimised oligonucleotide primers were designed and synthesised to enable the detection of the pCL68 SCV construct in transformed plants. The sequences of these primers were:

ATGLY002: CGTCTCGTGTGGTTATATTCA

ATGLY003: TCGATGCCTGAGATCTCAGCT

PCR mixtures which contained 5 μ l 10x Advantage Taq buffer; 5 μ l 2mM dNTPs; 0.5 μ l of primer ATGLY002 (100 μ M); 0.5 μ l of primer ATGLY003 (100 μ M); 5 μ l DNA template (*Arabidopsis thaliana* genomic DNA or control pCL68 SCV plasmid DNA); 0.25 μ l Advantage Taq polymerase; 33.75 μ l distilled water in a final volume of 50 μ l were set up. The PCR was carried out on a thermocycler using the following parameters: first a hot start at 94°C for 5 min, then 25 cycles consisting of 94 °C for 15 sec, 55 °C for 30 sec, and 72 °C for 3 min. The cycles were followed by 72 °C for 5 min and a final step of holding the samples at 8 °C.

A diagnostic DNA fragment of 977 bp was produced in these reactions.

The PCR results for pCL68 SCV transformed plants indicated that of the 30 of the 31 of the plants examined had successfully been transformed. Thus, all of the plants except for the plant labeled 1-005 contained the PGSIP gene.

EXAMPLE 15: Analysis of *Arabidopsis thaliana* Plants transformed with pCL76 SCV for the Presence of the PGSIP Downregulation Construct.

For the pCL76 SCV transformed lines a total of 10 kanamycin resistant plants were obtained. Leaf material was taken from regenerated *Arabidopsis thaliana* plants transformed with pCL76 and genomic DNA isolated. One leaf was excised from a plant growing in soil and placed in a 1.5ml eppendorf tube. The tissue was homogenised using a micropesle and 400 μ l extraction buffer (200mM Tris HCL pH 8.0; 250mM NaCl; 25mM EDTA; 0.5% SDS)

was added and ground again carefully to ensure thorough mixing. Samples were vortex mixed for approximately 5 seconds and then centrifuged at 10,000rpm for 5 minutes. A 350µl aliquot of the resulting supernatant was placed in a fresh eppendorf tube and 350µl chloroform was added. After mixing, the sample was allowed to stand for 5 minutes. This was then centrifuged at 10,000rpm for 5 minutes. A 300µl aliquot of the supernatant was removed into a fresh eppendorf tube. To this was added 300µl of propan-2-ol and mixed by inverting the eppendorf several times. The sample was allowed to stand for 10 minutes. The precipitated DNA was collected by centrifuging at 10,000rpm for 10 minutes. The supernatant was discarded and the pellet air dried. The pellet of DNA was resuspended in 50µl of distilled water and was used as a template in PCR.

PCR detection of PGSIP RNAi DNA

A pair of optimised oligonucleotide primers were designed and synthesised to enable the detection of the pCL76 SCV construct in transformed plants. The sequences of these primers were:

ATGLY001: TTTGAACAAACAAAAAGGTGGAAC

ATGLY002: CGTCTCGTCTGGTTATATTCA

PCR mixtures which contained 5 µl 10x Advantage Taq buffer; 5 µl 2mM dNTPs; 0.5 µl of primer ATGLY001 (100mM); 0.5 µl of primer ATGLY002 (100mM); 5 µl DNA template (*Arabidopsis thaliana* genomic DNA or control pCL76 SCV plasmid DNA); 0.25 µl Advantage Taq polymerase; 33.75 µl distilled water in a final volume of 50ml were set up. The PCR was carried out on a thermocycler using the following parameters: first a hot start at 94°C for 5 min, then 25 cycles of 94°C for 15 sec, 55°C for 30 sec, and 72°C for 3 min. The cycles are followed by 72°C for 5 min and the samples are then held at 8°C.

A diagnostic DNA fragment of 819 bp was produced in these reactions. Out of 8 kanamycin resistant plants tested, 2 were shown to contain the PGSIP RNAi gene construct.

EXAMPLE 16: Constitutive Overexpression and Downregulation of PGSIP Gene in Barley.

Starch is made in the leaves and the grain. To test the effect of overexpressing and downregulating the PGSIP gene in a monocot species, plasmids pCL68 SCV (sense construct) and pCL76 SCV (RNAi construct) were expressed in barley. These plasmids conferred constitutive expression as the genes were under the control of the double 35S promoter. Additionally, the full length gene and the RNAi cassette were expressed under the control of the rice actin promoter (US patent number 56141876). For this purpose, the Gateway cloning technology was used according to manufacturers instruction with slight modification (Invitrogen). The full length PGSIP was excised from plasmid pMC168 with NcoI-EcoRI and cloned into pENTR4 vector cut with NcoI-EcoRI resulting in plasmid called pMC175. The RNAi cassette was excised from plasmid pCL76 SCV with Sall-EcoICRI and cloned into pENTR1 vector cut with Sall-EcoRV resulting in plasmid pMC174. These plasmids were then recombined with Destination vector pWP492R12 SCV that contained the actin promoter flanked by two recombination sites (attR1 and attR2 on either side (Invitrogen). This resulted in plasmids pMC177 and pMC176 respectively which contained the PGSIP gene and the RNAi construct under the control of the rice actin promoter (US patent number 56141876). These plasmids are shown in Figs. 9 and 10.

The constructs were transformed into Agrobacterium strain (AGL-1) (Lazo et al., 1991, Bio/Technol 9: 963-967) for barley transformation. Immature embryos of the barley variety Golden Promise were transformed essentially according to the method of Tingay et al. (The Plant Journal 11(6): 1369-1376, 1997). Donor plants of Golden Promise were grown with an 18 hours day, and 18/13°C. Immature embryos (1.5 - 2.0 mm) were isolated and the axes removed. They were then dipped into an overnight liquid culture of Agrobacterium, blotted and transferred to co-cultivation medium. After 2 days the embryos were transferred to MS based callus induction medium with Asulam and Timentin for 10 days. Tissues were transferred at 2 weekly intervals, and at each transfer they were cut into small pieces and lined out on the plate. At the third transfer, only the embryogenic tissue was moved on to fresh medium. After a total of 8 weeks in culture, the tissue was transferred to regeneration

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medium (FHG), where plantlets formed within 2 - 4 weeks. These were transferred to Beatsons jars with growth regulator free medium until roots had formed, when they were transferred to Jiffies expandable teat pellets and then to the Conviron growth chambers.

The plants were analysed by PCR using following primers.

For plants containing pCL68 plasmid (sense expression)

5'- ATTGGAGAGGACAGCCCAAGC Glyc For
 5'- CTCCATCGTTGGATCTCGTTG-3' Glyc Rev (S)

For plants containing pCL76 plasmid (RNAi expression)

5'-ATTGGAGAGGACAGCCCAAGC-3' Glyc For

5'-GCGTCATCTCATCGCCAATCC – 3' Glyc Rev (D)

PCR was carried out as described in above

Results:

Six barley plants were regenerated after transformation with plasmid pCL68 SCV and eight plants with plasmid pCL76 SCV. The plants were first analysed by PCR and the leaves of the positive plants were subjected to iodine staining by Lugol. The results of PCR analysis are presented in Table 7.

Table 7. results of PCR screen of barley plants transformed with pCL68 SCV or pCL76 SCV.

| Construct | Plant no | PCR no. | PCR |
|-----------|----------|---------|-----|
| | Control1 | GG11 | Neg |
| | Control2 | GG12 | Neg |
| | Control3 | GG13 | Neg |
| pCL68 | 1 | GG1 | Pos |
| pCL68 | 2 | GG2 | Neg |

| | | | |
|-------|-----|------|-----|
| pCL68 | 3 | | |
| pCL68 | 4.1 | GG8 | Neg |
| pCL68 | 5.1 | | |
| pCL68 | 6.1 | GG3 | Neg |
| pCL68 | 6.2 | | |
| pCL68 | 6.3 | GG9 | Neg |
| pCL68 | 7.1 | GG10 | Neg |
| pCL76 | 1.1 | GG4 | Pos |
| pCL76 | 1.2 | GG5 | Pos |
| pCL76 | 1.3 | GG6 | Pos |
| pCL76 | 1.4 | GG14 | Pos |
| pCL76 | 1.5 | GG15 | Neg |
| pCL76 | 2 | GG7 | Neg |
| pCL76 | 3.1 | GG16 | Pos |
| pCL76 | 4.1 | GG17 | Neg |

One plant containing the sense construct was found to contain more starch granules in its leaves relative to control plants without the sense construct. The plants containing the RNAi construct were found to lack starch granules as shown in Figure 11A.

EXAMPLE 17: Seed Specific Overexpression and Downregulation of the PGSIP Gene in Barley

For seed specific expression, the plasmids pMC174 and pMC175 were recombined with the plasmid pWP491R12SCV that contained the seed specific promoter flanked by two recombination sites (attR1 and attR2 on either side (Invitrogen)). Barley plants were transformed according to the method of Tingay et al. (1997) with some modification as described for Example 13.

EXAMPLE 18: Analysis of Transformed *Solanum tuberosum* Plants for Presence of the PGSIP Construct

Analysis of regenerated Potato transformants.

Leaf material was taken from regenerated potato plants and genomic DNA isolated. One large potato leaf (approximately 30mg) was excised from an *in vitro* grown plant and placed in a 1.5ml eppendorf tube. The tissue was homogenised using a micropestle and 400 μ l extraction buffer (200mM Tris HCL pH 8.0; 250mM NaCl; 25mM EDTA; 0.5% SDS) was added and ground again carefully to ensure thorough mixing. Samples were vortex mixed for approximately 5 seconds and then centrifuged at 10,000rpm for 5 minutes. A 350 μ l aliquot of the resulting supernatant was placed in a fresh eppendorf tube and 350 μ l chloroform was added. After mixing, the sample was allowed to stand for 5 minutes. This was then centrifuged at 10,000rpm for 5 minutes. A 300 μ l aliquot of the supernatant was removed into a fresh eppendorf tube. To this was added 300 μ l of propan-2-ol and mixed by inverting the eppendorf several times. The sample was allowed to stand for 10 minutes. The precipitated DNA was collected by centrifuging at 10,000rpm for 10 minutes. The supernatant was discarded and the pellet air dried. The pellet of DNA was resuspended in 50 μ l of distilled water and was used as a template in PCR.

PCR mixtures which contained 5 μ l 10x Advantage Taq buffer; 5 μ l 2mM dNTPs; 0.5 μ l of either primer ATGLY001 or ATGLY003 (100 μ M); 0.5 μ l of primer ATGLY002 (100 μ M); 5 μ l DNA template (*Solanum tuberosum* c.v. Prairie genomic DNA, control pCL68 SCV plasmid DNA or control pCL76 SCV plasmid DNA); 0.25 μ l Advantage Taq polymerase; 33.75 μ l distilled water in a final volume of 50 μ l were set up. The PCR was carried out on a thermocycler using the following parameters: first a hot start at 94°C for 5 min, followed by 25 cycles of 94 ° C for 15 sec, 55 ° C for 30 sec, and 72 ° C for 3 min. The cycles were followed by 72 ° C for 5 min and a finally holding the temperature at 8 ° C.

A diagnostic DNA fragment of 977 bp was produced in these reactions from plasmid pCL68 SCV or 819 bp from plasmid pCL76 SCV. Lines of *Solanum tuberosum* c.v. Prairie transformed with pCL68 SCV or pCL76 SCV were tested by PCR and were shown to contain the construct.

Of 18 plants transformed with pCL68 SCV, all 18 contained the sense PGSIP construct. For the PGSIP RNAi construct (pCL76 SCV), 3 out of 8 plants contained the construct.

EXAMPLE 19: Analysis of Transformed Plants for PGSIP Expression.

Raising antisera to PGSIP proteins.

Expression of PGSIP proteins can be analysed by Western blotting. Antibodies to PGSIP are raised by inoculating rabbits with peptides corresponding to the *Arabidopsis thaliana* PGSIP protein sequences produced by expressing the sequence as a transcriptional fusion with glutathione-S-transferase in *E. coli* cells

Preparation of protein extracts.

Protein extracts from potato tuber were produced by taking up to 100mg of tissue and homogenising in 1ml of ice cold extraction buffer consisting of 50mM HEPES pH 7.5, 10mM EDTA, 10mM DTT. Additionally, protease inhibitors, such as PMSF or pepstatin were included to limit the rate of protein degradation. The extract was centrifuged at 13000 rpm for 1 minute and the supernatant decanted into a fresh eppendorf tube and stored on ice. The supernatants was assayed for soluble protein content using, for example, the BioRad dye-binding protein assay (Bradford, M.C. (1976) *Anal. Biochem.* 72, 248-254).

An aliquot of the soluble protein sample, containing between 10-50 μ g total protein was placed in an eppendorf tube and excess acetone (ca 1.5ml) added to precipitate the proteins which were collected by centrifuging the sample at 13000 rpm for 5 minutes. The acetone was decanted and the samples air-dried until all the residual acetone has evaporated.

SDS-polyacrylamide gel electrophoresis.

The protein samples were separated by SDS-PAGE. SDS PAGE loading buffer (2% (w/v) SDS; 12% (w/v) glycerol; 50 mM Tris-HCl pH 8.5; 5 mM DTT; 0.01% Serva blue G250) was added to the protein samples (up to 50 l). Samples were heated at 70°C for 10 minutes before loading onto a NuPage polyacrylamide gel. The electrophoresis conditions were 200 V constant for 1 hour on a 10% Bis-Tris precast polyacrylamide gel, using 50 mM MOPS, 50 mM Tris, 1 mM EDTA, 3.5 mM SDS, pH 7.7 running buffer, according to the NuPage methods

(Invitrogen, US 5,578,180).

Electroblotting.

Separated proteins were transferred from the acrylamide gel onto PVDF membrane by electroblotting (Transfer buffer: 20% methanol; 25 mM Bicine pH 7.2; 25 mM Bis-Tris, 1 mM EDTA, 50 μ M chlorobutanol) in a Novex blotting apparatus at 30 V for 1.5 hours.

Immunodetection.

After blocking the membrane with 5% milk powder in Tris buffered saline (TBS-Tween) (20mM Tris, pH 7.6; 140mM NaCl; 0.1% (v/v) Tween-20), the membrane was challenged with a rabbit anti-PGSIP antiserum at a suitable dilution in TBS-Tween. Specific cross-reacting proteins were detected using an anti-rabbit IgG-Horse radish peroxidase conjugate secondary antibody and visualised using the enhanced chemiluminescence (ECL) reaction (Amersham Pharmacia).

Detection of mRNA.

Expression of PGSIP mRNA was analysed in plants by rtPCR or by Northern blotting.

EXAMPLE 20: Analysis of Leaf Starch Content

Samples of leaves from control and transformed *Arabidopsis thaliana* plants which had been grown for 24 hours under high light (about 60 mg) were taken in a microfuge tube and extracted with 100 μ l of 45% HClO₄. This suspension was diluted with 1 ml of distilled water and centrifuged (14000 rpm, 2 min.) Aliquots of the extracts were then analysed for starch content by taking 100 μ l of the extract and mixing with an equal volume of Lugol's solution, the optical density of which was then measured at 540nm using a microplate reader. Standard starch mixtures were prepared in the same way and measured at the same time and the starch content of the extracts was calculated by reference to these standards.

Table 8. Starch contents of leaves of *Arabidopsis thaliana* plants transformed with pCL68 SCV (sense construct comprising SEQ ID NO: 1) compared with the starch contents of leaves of non

transformed (ncc) control plants. Control value is the mean \pm (the standard error of the mean) for three plants.

| samples | leaf starch content ug/g fresh weight (FWt). |
|---------|---|
| 37256 | 19.95 |
| 1-002 | 12.68 |
| 1-003 | 49.68 |
| 1-004 | 48.02 |
| 1-005 | 13.88 |
| 37407 | 17.47 |
| 37437 | 49.55 |
| 37468 | 24.88 |
| 37499 | 8.65 |
| 37529 | 17.71 |
| 37560 | 15.93 |
| 37590 | 9.95 |
| 37621 | 6.02 |
| 37257 | 21.9 |
| 37288 | 18.20 |
| 37316 | 11.82 |
| 37261 | 22.85 |
| 37381 | 9.51 |
| 37412 | 13.21 |
| 37442 | 33.60 |
| 37473 | 17.96 |
| 37504 | 8.88 |
| 37534 | 18.58 |
| 37565 | 11.98 |

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| | |
|-------|----------------------|
| 37295 | 32.83 |
| 37323 | 38.43 |
| 37354 | 16.16 |
| ncc | 22.59 (± 5.08) |

The ncc value represents the mean and standard error for the three control plants. Each data point otherwise represents a single leaf from an individual plant. Taking the error of the control as a measure of the population variation, then plants 1-003, 1-004, 1-007, 1-008, 6-007 and 9-003 have significantly more starch in their leaves than the controls. Plants 1-009, 1-012, 1-013, 2-003, 6-005, 6-009 and 6-011 have significantly lower starch contents. The copy number and level of expression of the sense construct in the plants are to be determined. The results demonstrate that a sense construct comprising SEQ ID NO: 1 can effectively alter the content of starch.

Table 9. Starch contents of leaves of *Arabidopsis thaliana* plants transformed with pCL76 SCV (RNAi construct) compared to controls.

| Samples | | starch content |
|-----------|------|----------------|
| | | μg per leaf |
| pCL76 SCV | 7 | 27.20 |
| pCL76 SCV | 20.1 | 26.96 |
| Control | ncc | 42.97 |

The data in these tables shows that the leaves of the transformed plants have an altered starch content compared to the untransformed controls (ncc).

EXAMPLE 21: Microscopic Analysis of Starch Granule Size and Number.

Starch granules were extracted from *Arabidopsis thaliana* or *Solanum tuberosum* tissue by taking 50-100 mg of tissue and homogenising in 1% sodium metabisulphite solution. After filtering the extract through miracloth, the starch was collected by centrifugation, 1300rpm for 5 minutes and then resuspended in 1 ml of water. Aliquots were taken and an equal amount of Lugol solution added to enhance the contrast of the starch granules. Suspensions were prepared for microscope imaging by placing onto a microscope slide. Representative micrographs were taken of the samples. The electronically captured images were then processed using suitable image analysis software, such as the package 'ImageJ'. This enabled a quantification of the size distributions of different starch samples to be made and compared.

Alternatively, samples of purified starch are either suspended in water and viewed with a light microscope or sputter -coated with gold and viewed with a scanning electron microscope such as a Phillips (Eindhoven, The Netherlands) XL30 Field Emission Gun scanning electron microscope at 3kV.

Starch granules can be examined in tissues as well. For example, starch in tissues is stained using Lugol's solution (1% Lugol's solution, I-KI [1:2, v/v]; Merck). Starch can then be examined, for example, in longitudinal sections of tubers. Alternatively the starch can be further isolated subsequent to staining and suspended in water, and stained again with a few drops of Lungol's solution and examined microscopically.

The radii of the blue staining core of the starch granules and the total granule are measured microscopically using an ocular micrometer. If granules are ovoid in shape, both long radius and short radius measurements are taken. The radii of the blue-staining core and the total granule are determined by measuring individual, randomly chosen starch granules.

EXAMPLE 22: Analysis of Starch Functionality.*Preparation of starch.*

Starch was extracted from potato tubers by taking 0.5-1 kg of washed tuber tissue and homogenising using a juicerator chased with 200ml of 1% Sodium bisulphite solution. The starch was allowed to settle, the supernatant decanted off and the starch washed by

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resuspending in 200 ml of ice-cold water. The resulting starch pellet was left to air dry. Once dried the starch was stored at -20 °C.

Alternatively, other methods can be utilized to isolate starch, for example, samples of tubers are first homogenized in extraction buffer (10 mM EDTA, 50 mM Tris, pH 7.5, 1mM DTT, 0.1% Na2S2O5). The resulting fibrous substance is then washed several times with the extraction buffer and filtered. The filtrate is allowed to set at 4 °C and the supernatant is discarded after the starch granules have settled. Starch granules are then washed with extraction buffer, water, and acetone and dried at 4 °C.

With maize and other cereal crops, seeds are soaked in 50ml of a 20 mM sodium acetate, pH 6.5, 10 mM mercuric chloride solution. After 24 hr, the germ and pericarp are removed and 50 ml of fresh solution is added for an additional 24 hr. Endosperm is repeatedly homogenized for 1 minute intervals in a mortar and pestle, and freed starch granules are purified by multiple extractions with saline and toluene (Boyer et al., 1976, Cereal Chemistry 53: 327-337). Granular starch is washed three times with double distilled water, once with acetone, and dried at 40 °C.

Viscometric analysis of starch.

Starch samples were analysed for functionality by testing rheological properties using viscometric analysis (rapid visco analyzer (RVA) or differential scanning calorimetry (DSC)). Viscosity of starches can also be measured by various other techniques. For example, a Rapid Visco Analyser Series 4 instrument (Newport Scientific, Sydney Australia) can be utilized with a 13 min profile where 2 g of starch are analyzed in water at a concentration of 7.4% (w/v) and the analysis used the stirring and heating protocol that suggested by Newport Scientific. For longer profiles, 2.5 g starch samples are used at a concentration of 10% (w/v). The sample is heated while stirring at $1.5 \text{ }^{\circ}\text{C min}^{-1}$ from 50 °C to 95 °C for 15 min then cooled to 50 °C at 15 min^{-1} . Viscosity is measured in centipose (cP).

EXAMPLE 23: Analysis of Fine Structure of Starch

Amylopectin chain length distribution

One method for examining the fine structure of starch is ^{14}C labeling of amylopectin chains to determine chain lengths. Extracted starch granules are suspended at 25 mg ml $^{-1}$ in medium comprising 100 mM Bicine (pH 8.50, 25 mM potassium acetate, 10 mM DTT, 5 mM EDTA, 1 mM ADP[U- ^{14}C] glucose at 18.5 GBq mol $^{-1}$ and 10 μl starch suspension in a total volume of 100 μl , for each sample. Samples are then incubated for 1 hour at 25 °C. The incubation is terminated by addition of 3 ml 750 ml $^{-1}$ aqueous methanol containing 10 g l-1 KCL (methanol/KCL). After incubation for at least 5 minutes at room temperature, starch is collected by centrifugation at 2000 g for 5 min. The supernatant is discarded and the pellet is resuspended in 0.3 ml distilled water. The Methanol/KCL wash, centrifugation, and resuspension are repeated 2-4 times. The resulting pellets are dried at room temperature, dissolved with 50 μl 1M NaOH, and diluted with 50 μl distilled water. To determine the average length of amylopectin chains into which ^{14}C was incorporated, products of incubation with ADP[U- ^{14}C] glucose are debranched with isoamylase and subjected to chromatography on a column of Sepharose CL-4B. The glucan eluting earlier from the column consists of longer chains than glucan eluting later from the column.

Another method for examining the fine structure of starch is chromatography without labeling. A 10 mg sample of isolated starch is dissolved in 100 μl 0.1 M NaOH for 1 hour at 95 °C. The sample is diluted in 900 μl water, 150 μl 1 M sodium citrate (pH 5.0). The starch is then debranched by adding 300 units of isoamylase, or hydrolysed with 300 units of alpha-amylase, or beta-amylase for 24 hours at 37 °C. A 100 μl aliquot sample of the hydrolysed samples is analyzed with chromatography. For example HPAE-PAD chromatography (Carbo PAC PA-100 column; Dionex, Idstein, Germany; flow 1 ml min $^{-1}$; buffer A: 150 mM NaOH; buffer B: 1 M sodium acetate in buffer A) with an applied gradient comprising 0-5 min 100% A; 5-20 min 85% A, 15% B, 20-35 min 70% A, 30% B (linear); 35-80 min 50% A, 50% B (convex).

Alternatively, HPLC chromatography is utilized, where partially hydrolyzed debranched starch samples in 0.01 N NaOH (5 mg/ml), and 2 ml are applied to a size exclusion column (Sephadex G-75, 1.5 X 100cm). The mobile phase is 0.01 N NaOH and

the flow rate is 0.6-0.9 ml/min. Samples are analyzed for total carbohydrate by the phenol-sulfuric acid test (Hodge and Hofreiter, 1962, Vol. 1, R.L. Whistler and ML Wolfrom (Eds.), Corporation. Version 7. Academic Press, New York, pp: 388-389) and the Park Johnson test for reduced ends (Porro et al., 1981, *Anal Biochem.* 118(2):301-6). Based on these two analyses the average chain length for each fraction is calculated.

Amylopectin is further characterized by measuring the low molecular weight to high molecular weight chain ratio (on a weight basis) according to the method of Hizukuri (Hizukuri, 1986, *Carbohydrate Research*, 147, 342-347).

An alternative method for analyzing amylopectin chains is gel electrophoresis. Starch samples are debranched with isoamylase, derivatised with fluorophore APTS, and subjected to gel electrophoresis in an Applied Biosystem DNA sequencer. Data are analyzed by Genescan software. The method allows for identification of authentic maltohexaose and maltoheptaose as well as a determination of percent molar differences and the degree of polymerization, distribution of chain lengths, between samples.

Amylose content of starch

Amylose percentages are determined by gel permeation chromatography according to Denyer et al. (Denyer et al., 1995, *Plant Cell Environ* 18:1019-1026) or by gel filtration analysis according to Boyer and Liu (Boyer and Liu, 1985, *Starch Starke* 37:73-79).

Alternatively, the amylose contents are determined spectrophotometrically in 1 to 2 mg isolated starch according to the iodometric method described by Hovenkamp-Hermelink et al. 1988. Amperometric titrations are performed according to Williams et al 1970 to determine the average amylose content per sample.

EXAMPLE 24: cDNA Isolation From Barley

A database search using the *Arabidopsis* genes AT3g18660 and at1g77130, against an in-house database identified two barley sequences. The accessions corresponding to Genbank: BE438665 and Genbank: BE438754 showed significant similarity to the *Arabidopsis* PGSlP genes (9e-34). The sequences called Barley SEQ1 and Barley SEQ2 are shown in SEQ ID Nos: 16 and 18.

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All publications, patents and patent applications mentioned in this specification are herein incorporated by reference into the specification to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference

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CLAIMS

1. An isolated nucleic acid molecule that:
 - (i) comprises a nucleotide sequence which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: 3, or a fragment thereof;
 - (ii) comprises a nucleotide sequence at least 40% identical to SEQ ID NOs: 1 or 2, or a complement thereof; or
 - (iii) hybridizes to a nucleic acid molecule consisting of SEQ ID NOs: 1 or 2 under low stringency conditions of hybridization, or a complement thereof.
2. The isolated nucleic acid molecule of claim 1, wherein the nucleic acid molecule comprises SEQ ID NOs: 1 or 2 or a complement thereof.
3. The isolated nucleic acid molecule of claim 1, comprising a nucleotide sequence selected from the group consisting of nucleotide residues 516-592, 681 to 918, 1039 to 1655, 1762 to 2536, and 2991 to 3264 of SEQ ID NO: 1.
4. An isolated nucleic acid molecule that:
 - (i) comprises a nucleotide sequence which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: 11, or a fragment thereof;
 - (ii) comprises a nucleotide sequence at least 70% identical to SEQ ID NO: 10, or a complement thereof, wherein the nucleotide sequence does not encode the amino acid of SEQ ID NO: 35; or
 - (iii) hybridizes to a nucleic acid molecule consisting of SEQ ID NO: 10 under stringent conditions of hybridization, or a complement thereof, wherein the nucleotide sequence does not encode the amino acid of SEQ ID NO: 35.
5. The isolated nucleic acid molecule of claim 4, wherein the nucleic acid molecule comprises SEQ ID NO: 10 or a complement thereof.

6. An isolated nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence that is at least 98% identical to SEQ ID NO: 9.
7. An isolated nucleic acid molecule thereof comprising the nucleotide sequence of SEQ ID NO: 8 or a complement thereof.
8. An isolated nucleic acid molecule that:
 - (i) comprises a nucleotide sequence which encodes a polypeptide comprising the amino acid sequence of SEQ ID NOs: 7, 13, 15, 17, 19, 21, 22, 24, 26, 28, 30, 32, 34, or a fragment thereof;
 - (ii) comprises a nucleotide sequence at least 70% identical to SEQ ID NOs: 4, 5, 6, 12, 14, 16, 18, 20, 23, 25, 27, 29, 31, 33, or a complement thereof; or
 - (iii) hybridizes to a nucleic acid molecule consisting of SEQ ID NOs: 4, 5, 6, 12, 14, 16, 18, 20, 23, 25, 27, 29, 31, 33 under stringent conditions of hybridization, or a complement thereof.
9. The isolated nucleic acid molecule of claim 8, wherein the nucleic acid molecule comprises SEQ ID NOs: 4, 5, 6, 12, 14, 16, 18, 20, 23, 25, 27, 29, 31, 33, or a complement thereof.
10. A fragment of the isolated nucleic acid molecule of any one of claims 1-9, wherein the fragment comprises at least 40, 60, 80, 100 or 150 contiguous nucleotides of the nucleic acid molecule.
11. The isolated nucleic acid molecule of claim 1 comprising the nucleotide sequence of nucleotides 1-195 of SEQ ID NO: 2, or a complement thereof.
12. An isolated polypeptide comprising the amino acid sequence of amino acid residues 1-65 of SEQ ID NO: 3, or a fragment thereof.

13. An isolated polypeptide comprising:
 - (i) an amino acid sequence that is at least 70% identical to SEQ ID NO: 3 or a fragment thereof;
 - (ii) an amino acid sequence encoded by the nucleic acid molecule of claim 1; or
 - (iii) an amino acid sequence of SEQ ID NO: 3.
14. An isolated polypeptide comprising:
 - (i) an amino acid sequence at least 70% identical to SEQ ID NO: 11, or a fragment thereof;
 - (ii) an amino acid sequence encoded by the nucleic acid molecule of claim 4; or
 - (iii) an amino acid sequence of SEQ ID NO: 11.
15. An isolated polypeptide comprising:
 - (i) an amino acid sequence that is at least 98% identical to SEQ ID NO: 9;
 - (iii) an amino acid sequence encoded by the nucleic acid molecule of SEQ ID NO: 8, or a complement thereof; or
 - (v) an amino acid sequence of SEQ ID NO: 9, or a fragment thereof.
16. An isolated polypeptide comprising:
 - (i) an amino acid sequence that is at least 70% identical to SEQ ID NOs: 7, 13, 15, 17, 19, 21, 22, 24, 26, 28, 30, 32, 34, or a fragment thereof;
 - (ii) an amino acid sequence encoded by the nucleic acid molecule of claim 8;
 - (iii) an amino acid sequence of SEQ ID NOs: 7, 13, 15, 17, 19, 21, 22, 24, 26, 28, 30, 32, 34.

17. A fragment of a polypeptide comprising at least 5 amino acid residues, wherein said fragment is a portion of the polypeptide encoded by a nucleic acid molecule selected from the group consisting of exon I, exon II, exon III, exon IV and exon V of SEQ ID NO: 1.

18. A polypeptide comprising the amino acid sequence of SEQ ID: 3, 7, 9, 11, 13, 15, 17, 19, 21, 22, 24, 26, 28, 30, 32, 34 which further comprising one or more conservative amino acid substitution.

19. A fusion protein comprising the amino acid sequence of any one of claims 12-18 and a heterologous polypeptide.

20. A fragment or immunogenic fragment of a polypeptide of any one of claims 12-18, wherein the fragment comprises at least 5, 8, 10, 15, 20, 25, 30 or 35 consecutive amino acids of the polypeptide.

21. An antibody that immunospecifically binds to a polypeptide of any one of the claims 12-18.

22. A method for making a polypeptide of any one of the claims 12-18, comprising the steps of:

- culturing a cell comprising a recombinant polynucleotide encoding the polypeptide of any one of claims 12-18 under conditions that allow said polypeptide to be expressed by said cell; and
- recovering the expressed polypeptide.

23. A complex comprising a polypeptide encoded by a nucleic acid molecule of any of claims 1-9 and a starch molecule.

24. The complex of claim 23, wherein the starch molecule comprises from 1 to 700 glucose units.

25. The complex of claim 23, wherein the starch molecule comprises branching chains of glucose polysaccharides.

26. A vector comprising a nucleic acid molecule of any one of claims 1-9.

27. An expression vector comprising a nucleic acid molecule of any one of claims 1-9 and at least one regulatory region operably linked to the nucleic acid molecule.

28. The expression vector of claim 27, wherein the regulatory region confers chemically-inducible, dark-inducible, developmentally regulated, developmental-stage specific, wound-induced, environmental factor-regulated, organ-specific, cell-specific, and/or tissue-specific expression of the nucleic acid molecule or constitutive expression of the nucleic acid molecule.

29. The expression vector of claim 27, wherein the regulatory region is selected from the group consisting of a 35S CaMV promoter, a rice actin promoter, a patatin promoter, and a high molecular weight glutenin gene of wheat.

30. An expression vector comprising the antisense sequence of a nucleic acid molecules of any one of claims 1-9, wherein the antisense sequence is operably linked to at least one regulatory region.

31. A genetically-engineered cell which comprises a nucleic acid molecule of any one of claims 1-9.

32. A cell comprising the expression vector of claim 27.

33. A cell comprising the expression vector of claim 30.
34. A genetically-engineered plant comprising the isolated nucleic acid molecule of any of claims 1-9.
35. The genetically-engineered plant of claim 34 and progeny thereof, further comprising a transgene encoding an antisense nucleotide sequence.
36. The genetically-engineered plant of claim 31, further comprising an RNA interference construct.
37. A cell comprising an a 35SCaMV constitutive promoter operably linked to a nucleic acid molecule of SEQ ID NO:2 or a rice actin promoter operably linked to an RNA interference construct comprising fragments of a nucleic acid molecule of SEQ ID NO:2, wherein said promoter confers expression of said fragments.
38. A method of altering starch synthesis in a plant comprising introducing into a plant:
 - (i) a nucleic acid sequence comprising a starch primer gene, or a fragment thereof;
 - (ii) a nucleotide sequence that hybridises under stringent conditions to a sequence of (i) or its complement; or
 - (iii) an agent which is capable of altering the expression of a sequence of (i) or (ii);such that starch synthesis is altered relative to a plant without any of the above sequences.

39. A method of altering starch synthesis in a plant comprising, introducing into a plant an expression vector of claim 27, such that starch synthesis is altered relative to a plant without the expression vector.

40. A method of altering starch synthesis in a plant comprising, introducing into a plant at least an expression vector of claim 30, such that starch synthesis is altered in comparison to a plant without the expression vector.

41. A method of altering starch granules in a plant comprising, introducing into a plant at least an expression vector of claim 27, such that the starch granules are altered in comparison to a plant without the expression vector.

42. A method of altering starch granules in a plant comprising, introducing into a plant at least an expression vector of claim 30, such that the starch granules are altered in comparison to a plant without the expression vector.

43. The method of claim 42, wherein starch granules are absent from leaves of the plant comprising at least an expression vector.

44. A plant part comprising a nucleic acid molecule of any of claims 1-9 or a nucleic acid of the method of claim 38, wherein starch synthesis is altered.

45. The plant part of claim 44, wherein the part is a tuber, seed or leaf.

46. The modified starch obtained from the plant parts of claim 44, wherein the modification is selected from the group consisting of a ratio of amylose to amylopectin, amylose content, size of starch granules, quantity of size of starch granules, a ratio of small to large starch granules, and rheological properties of the starch as measured using viscometric analysis.

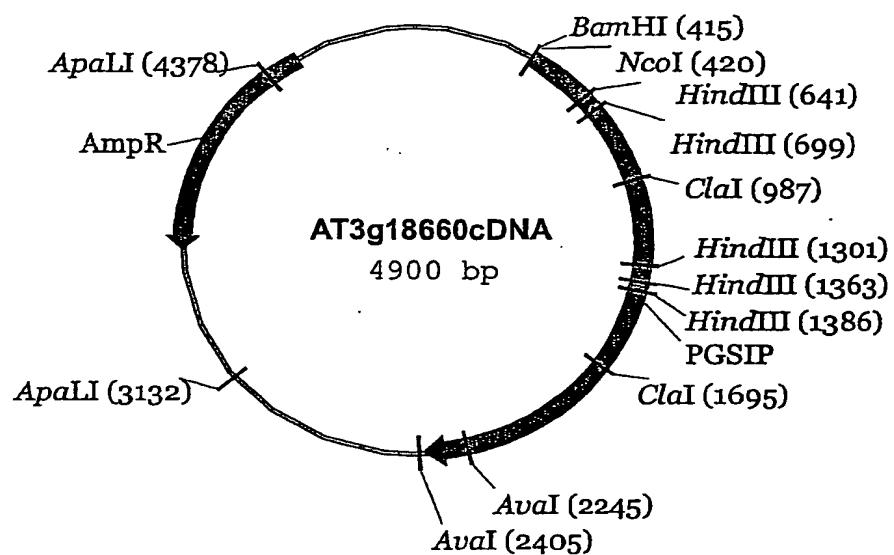


Figure 1

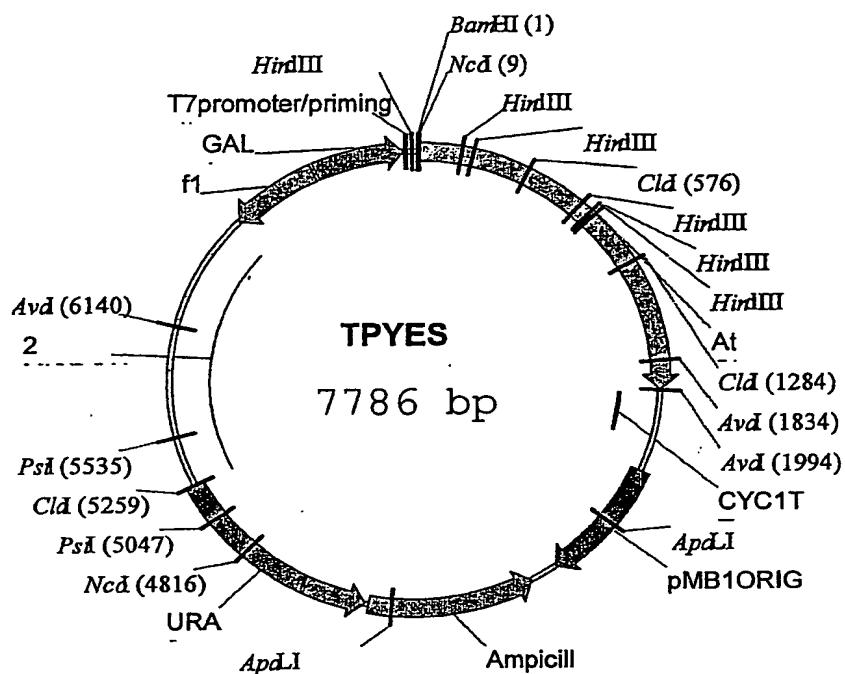


Figure 2

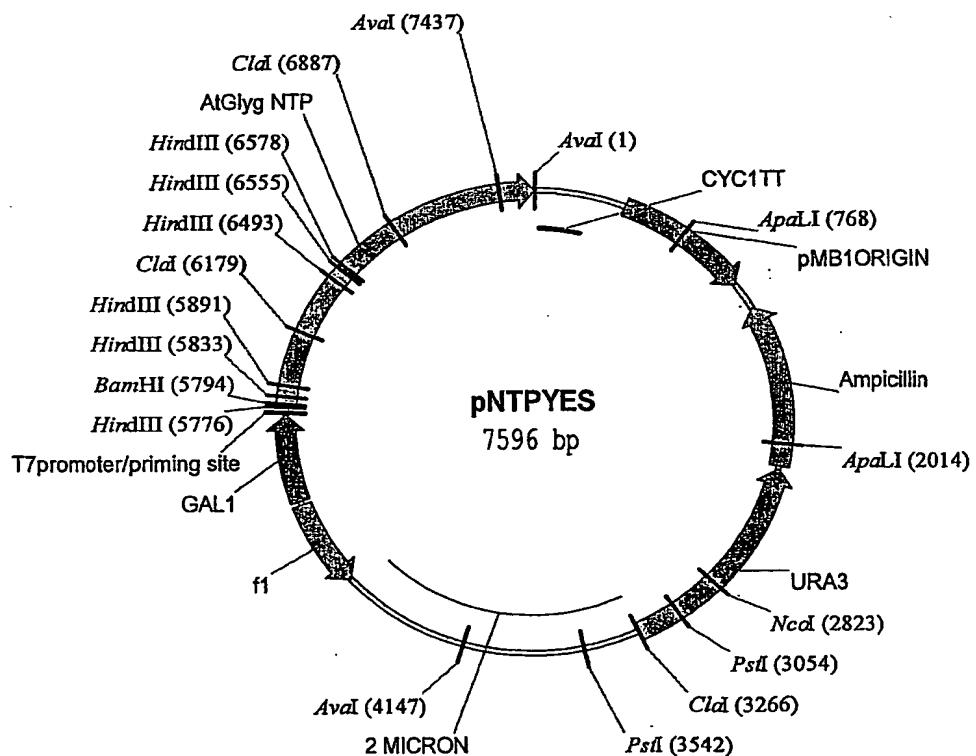


Figure 3

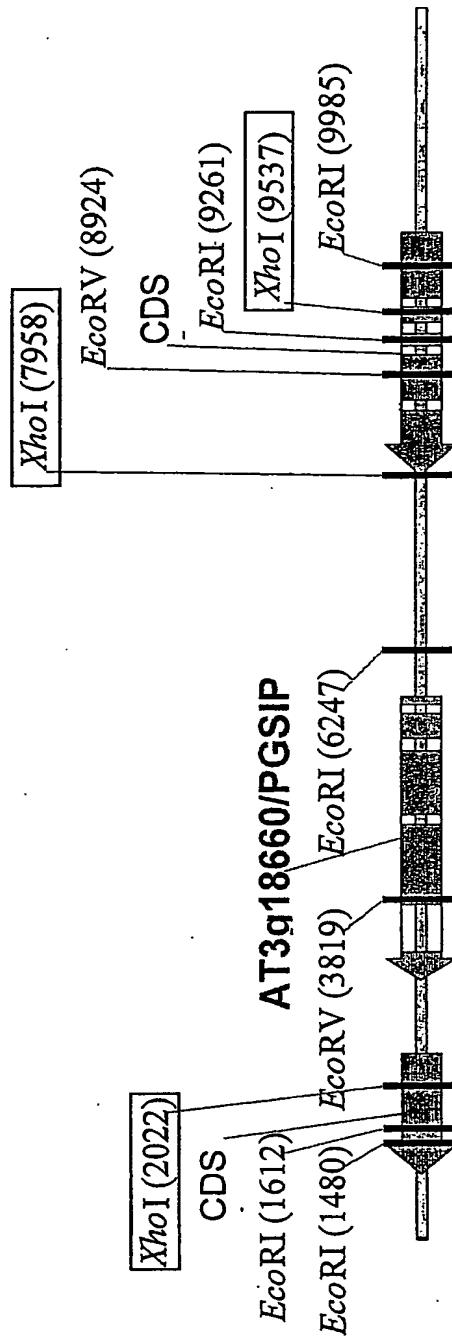


Figure 4A

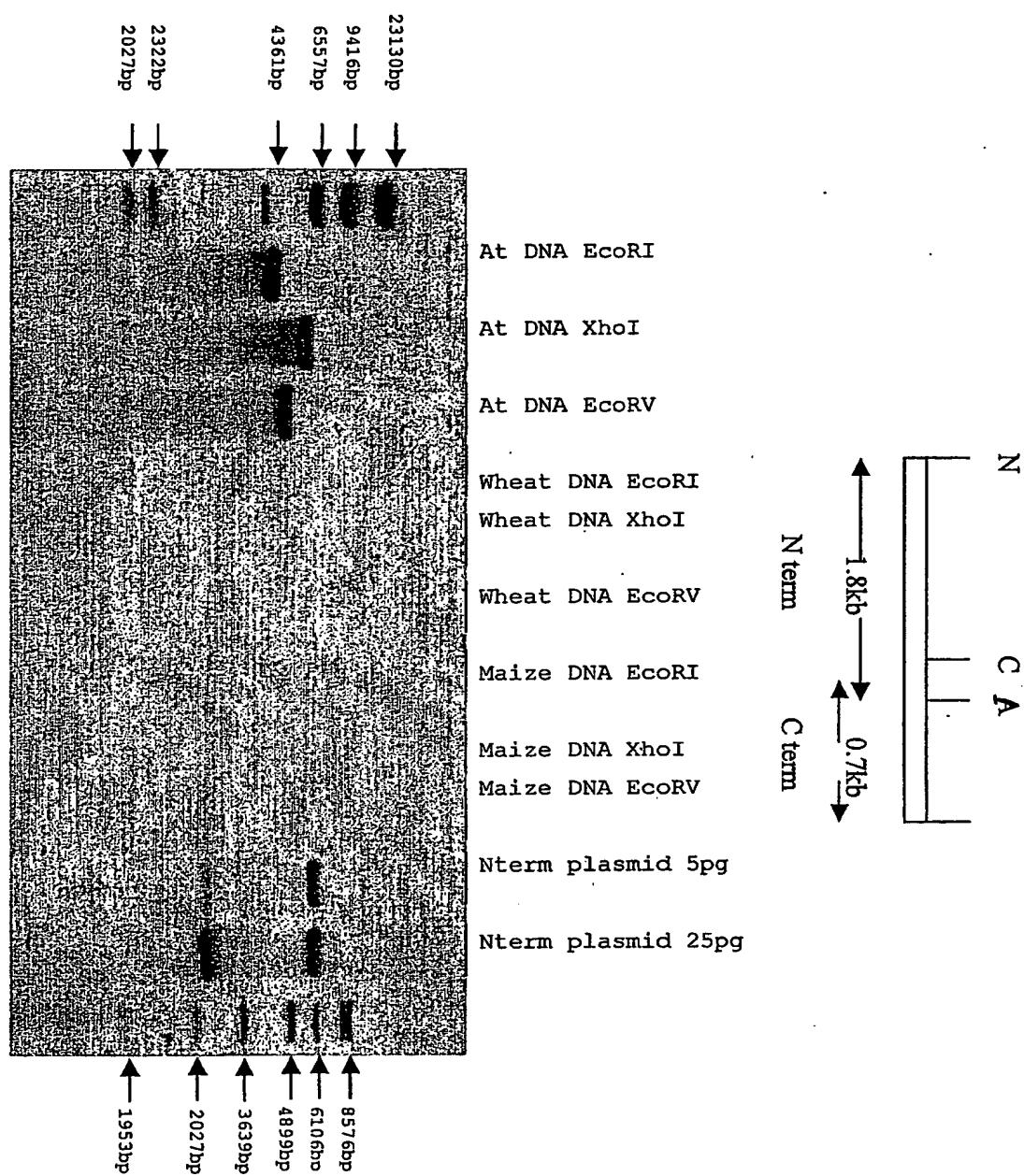


Figure 4B

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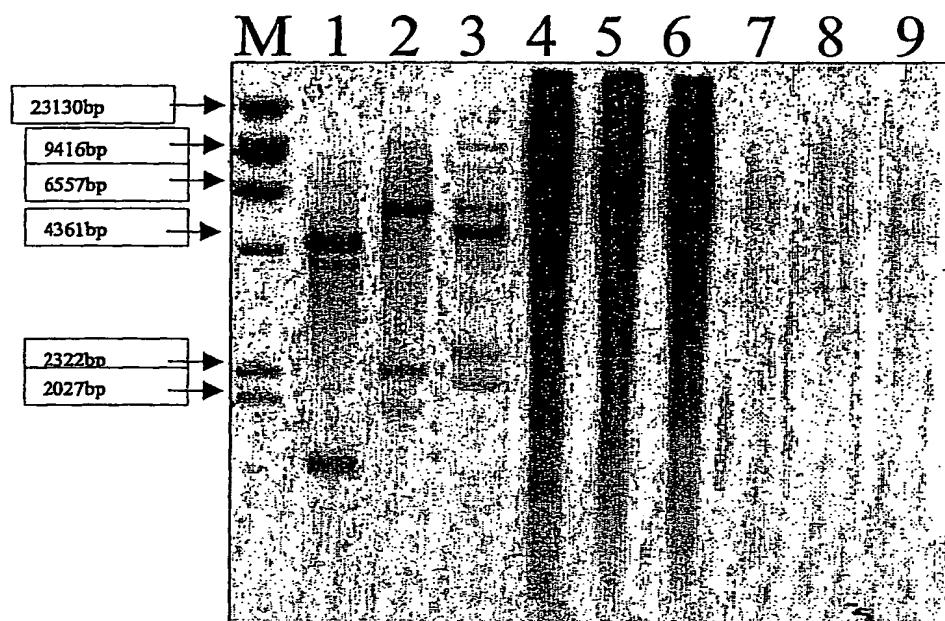


Figure 5A

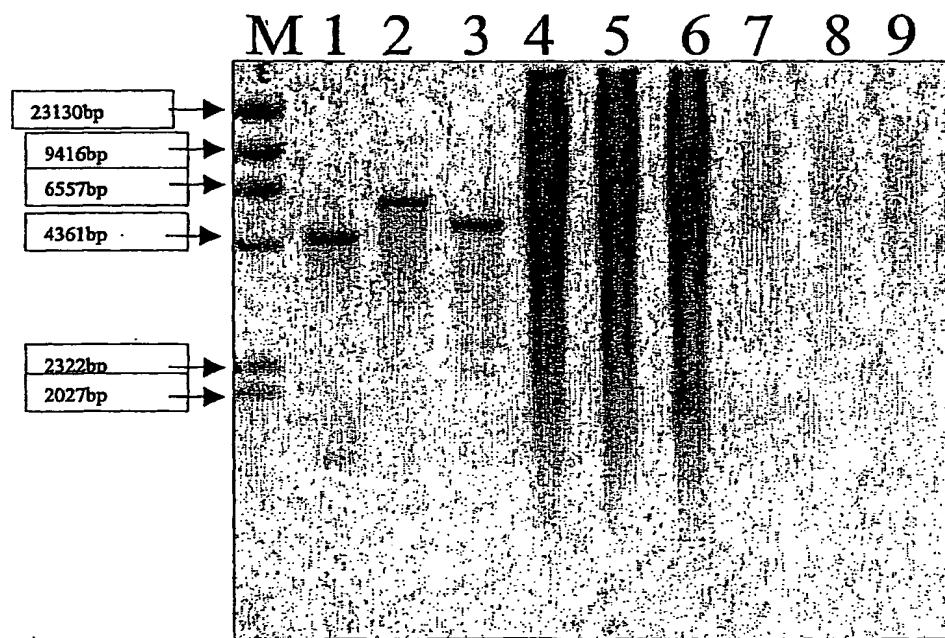


Figure 5B

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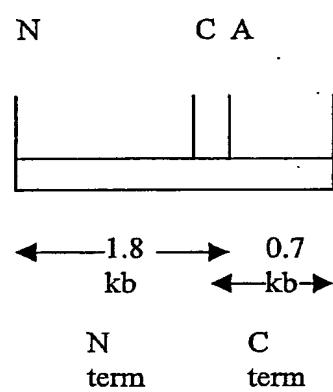
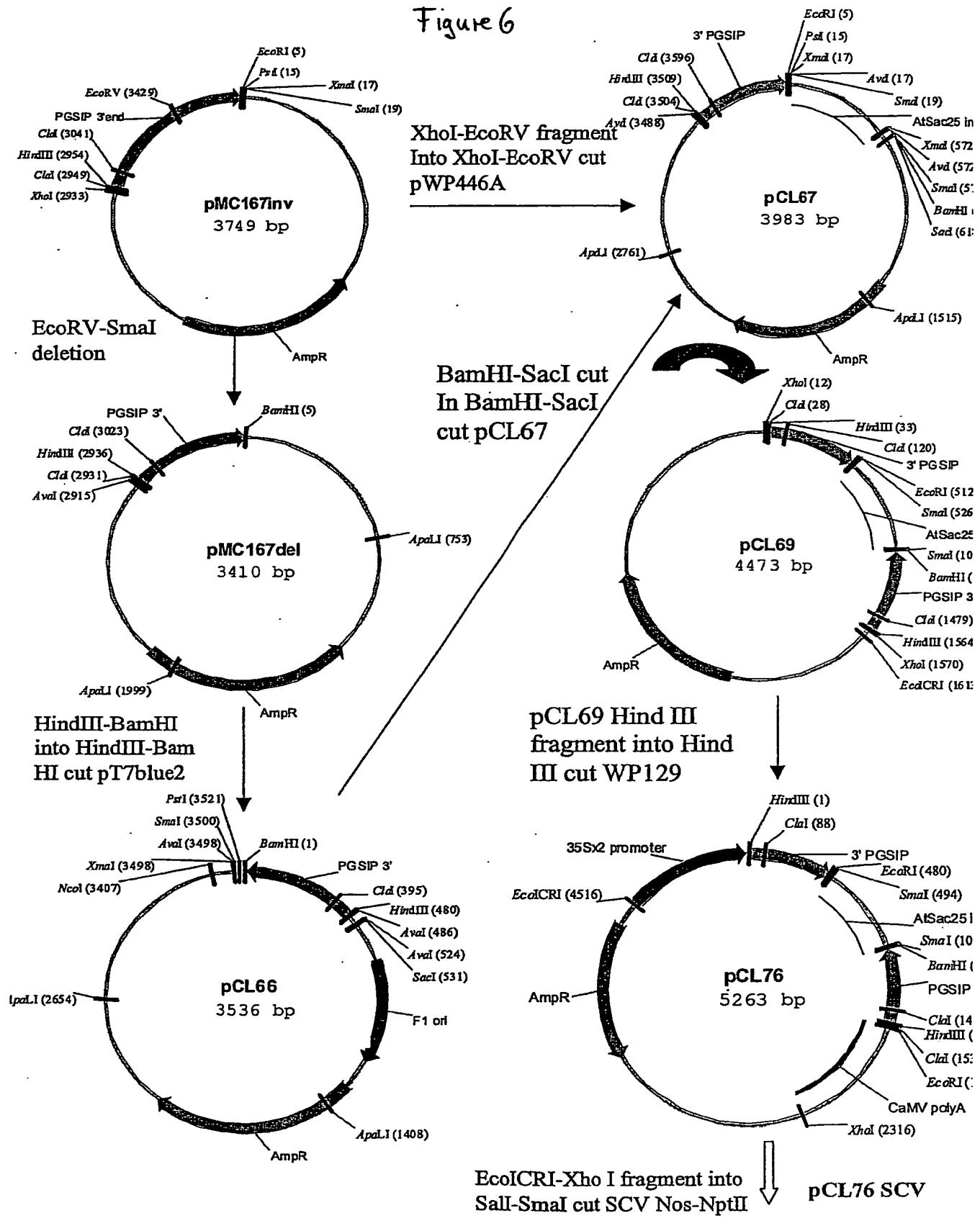


Figure 5C

Figure 6



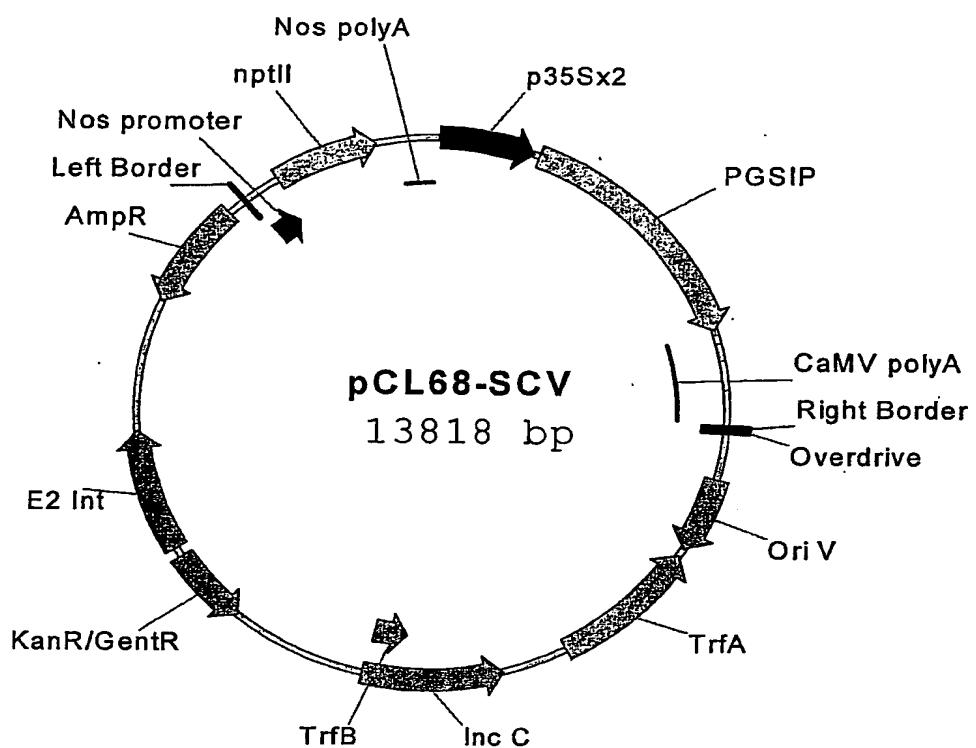


Figure 7

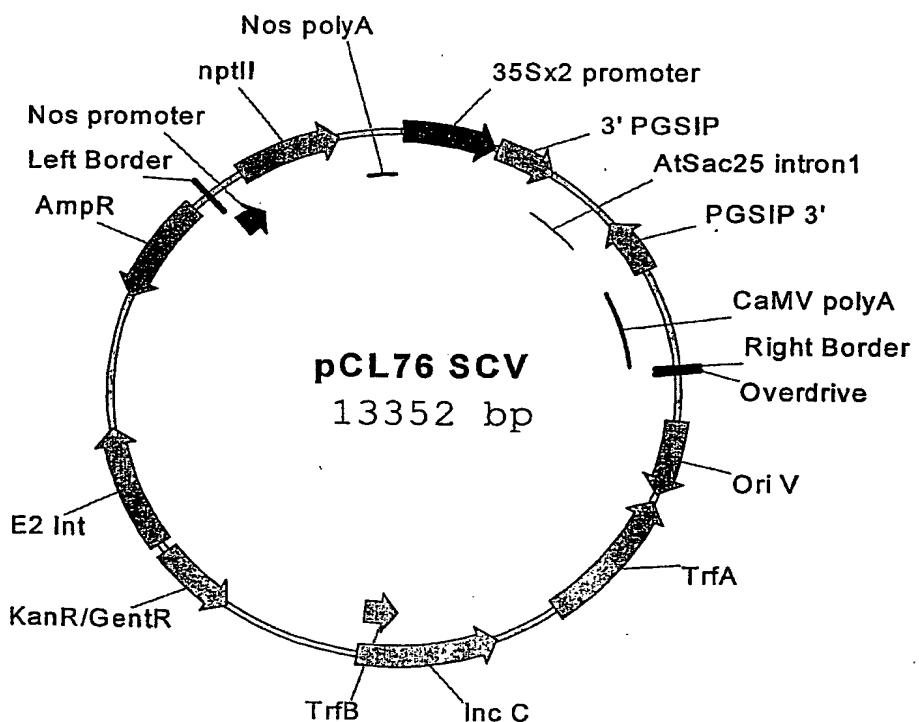


Figure 8

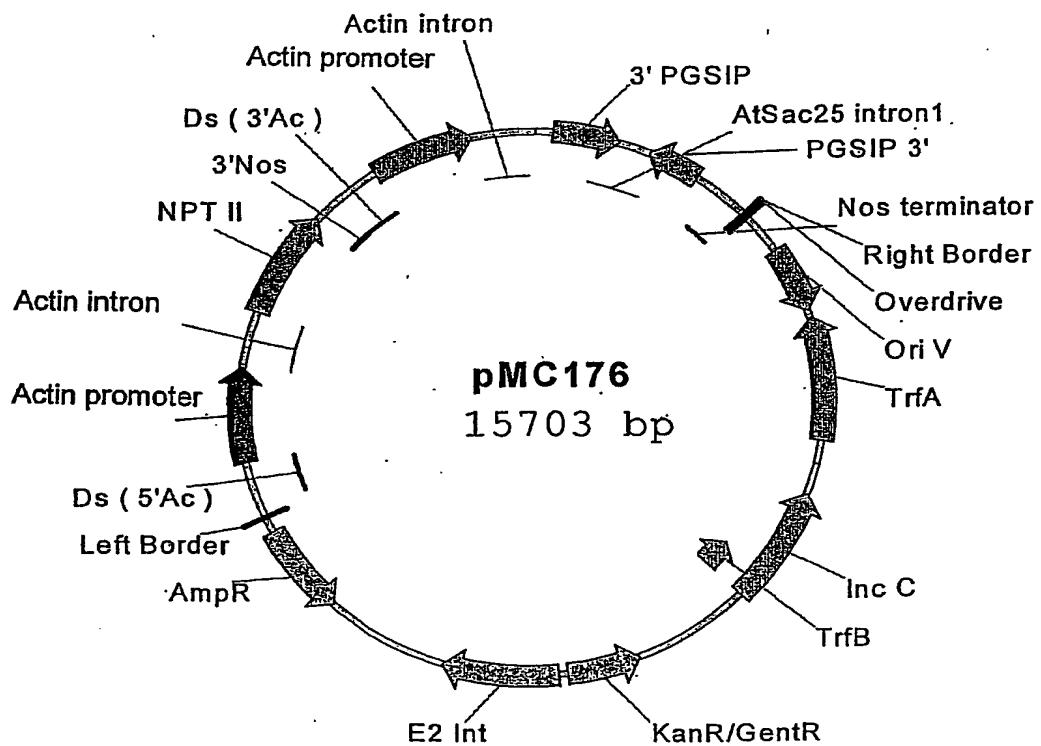
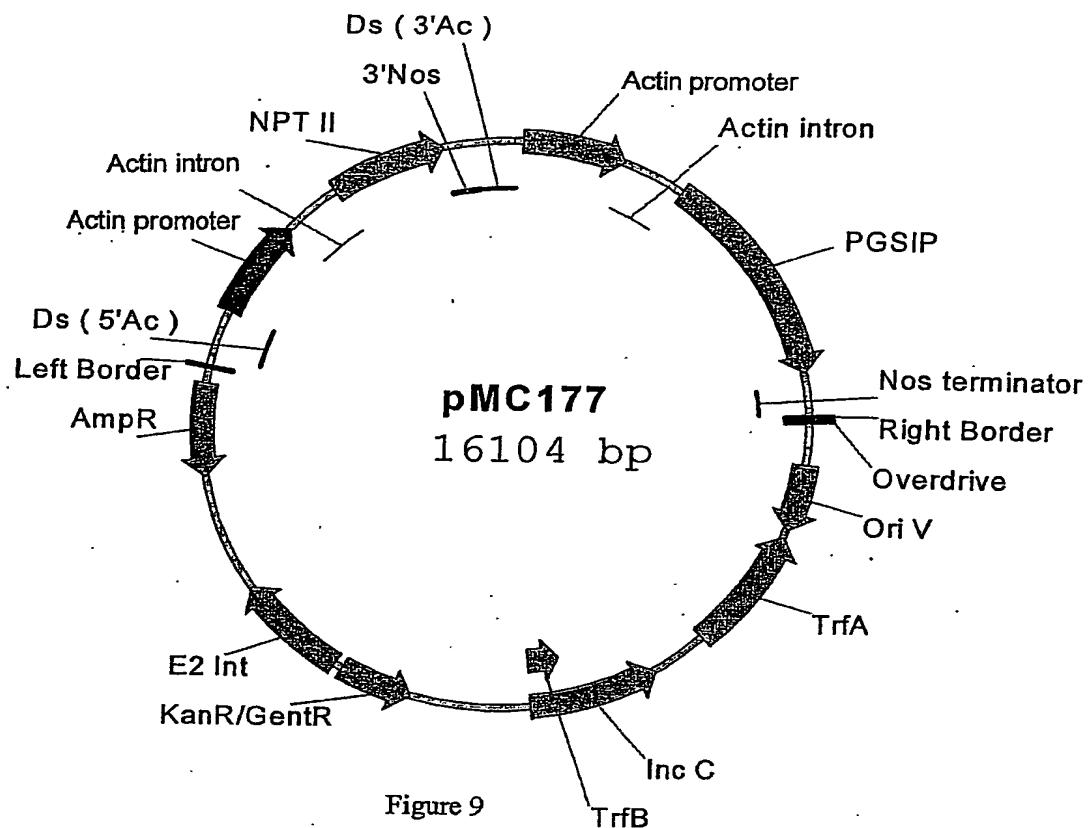




Figure 11A.

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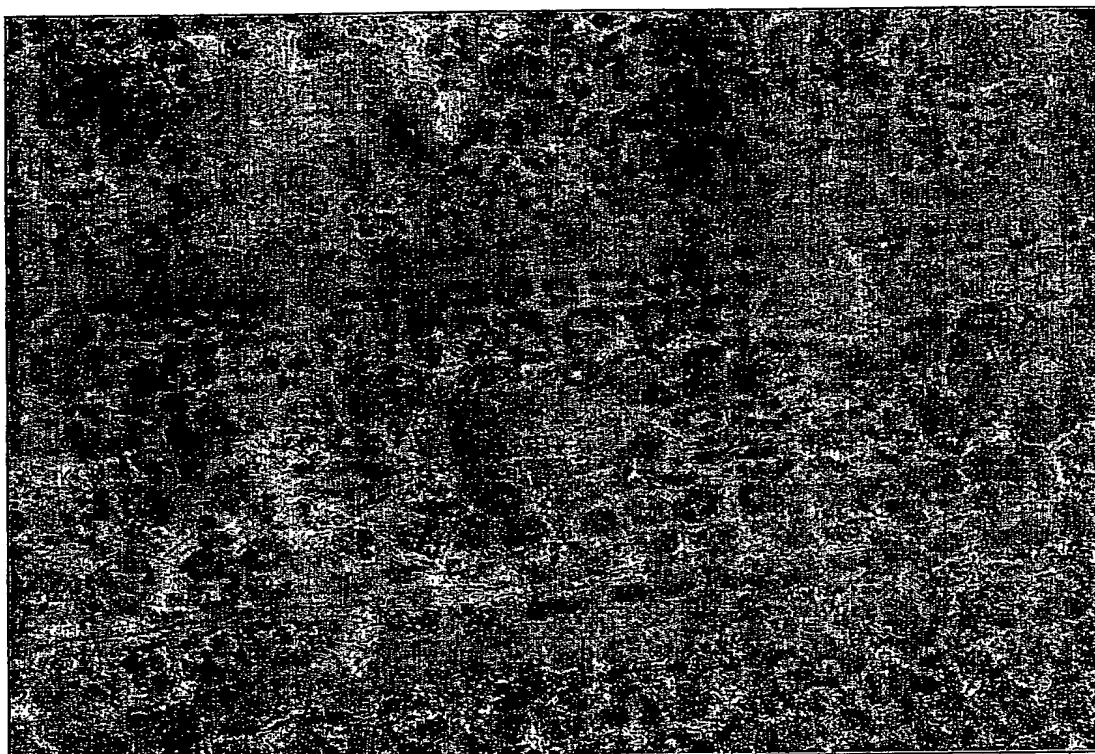


Figure 11B.

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| | | | | | | | | | | | | | | | |
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| ctg | gtt | atg | ttc | aca | ctc | tac | tgt | tct | cca | ccg | ttg | caa | att | cct | 96 |
| Ieu | Val | Ala | Met | Phe | Thr | Leu | Tyr | Cys | Ser | Pro | Pro | Leu | Gln | Ile | Pro |
| | | | | | | | | | | | | | | 20 | |
| | | | | | | | | | | | | | | 25 | |
| | | | | | | | | | | | | | | 30 | |

| | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| gaa | gat | cca | tca | agt | ttt | gca | aac | aaa | tgg | ata | cta | gaa | cct | gct | gta | 144 |
| Glu | Asp | Pro | Ser | Ser | Phe | Ala | Asn | Lys | Trp | Ile | Ieu | Glu | Pro | Ala | Val | |
| | | | | | | | | | | | | | 35 | 40 | 45 | |

| | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| acc | aca | gat | cct | cgc | tat | ata | gct | aca | tct | gag | atc | aac | tgg | aac | agt | 192 |
| Thr | Thr | Asp | Pro | Arg | Tyr | Ile | Ala | Thr | Ser | Glu | Ile | Asn | Trp | Asn | Ser | |
| | | | | | | | | | | | | | 50 | 55 | 60 | |

| | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| atg | tca | ctt | gtt | gtt | gag | cat | tac | tta | tct | ggc | aga | agc | gag | tat | caa | 240 |
| Met | Ser | Leu | Val | Val | Glu | His | Tyr | Leu | Ser | Gly | Arg | Ser | Glu | Tyr | Gln | |
| | | | | | | | | | | | | | 65 | 70 | 75 | 80 |

| | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| gga | att | ggc | ttt | cta | aat | ctc | aac | gat | aac | gag | att | aat | cga | tgg | cag | 288 |
| Gly | Ile | Gly | Phe | Leu | Asn | Leu | Asn | Asp | Asn | Glu | Ile | Asn | Arg | Trp | Gln | |
| | | | | | | | | | | | | | 85 | 90 | 95 | |

| | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| gtg | gtc | ata | aaa | tct | cac | tgt | cag | cat | ata | gct | ttg | cat | cta | gac | cat | 336 |
| Val | Val | Ile | Lys | Ser | His | Cys | Gln | His | Ile | Ala | Leu | His | Leu | Asp | His | |

| 100 | 105 | 110 | | |
|---|-----|-----|-----|-----|
| 10 | | | | |
| gct gca agt aac ata act tgg aaa tct tta tac ccg gaa tgg att gac Ala Ala Ser Asn Ile Thr Trp Lys Ser Leu Tyr Pro Glu Trp Ile Asp 115 | 120 | 125 | 384 | |
| gag gaa gaa aaa ttc aaa gtc ccc act tgt cct tct ctt cct tgg att Glu Glu Glu Lys Phe Lys Val Pro Thr Cys Pro Ser Leu Pro Trp Ile 130 135 140 | | | | 432 |
| caa gtt cct gac aag tct cga atc gat ctt atc att gcc aag ctc cca Gln Val Pro Asp Lys Ser Arg Ile Asp Leu Ile Ile Ala Lys Leu Pro 145 150 155 160 | | | 480 | |
| tgt aac aag tca gga aaa tgg tca aga gat gtg gct aga ttg cac tta Cys Asn Lys Ser Gly Lys Trp Ser Arg Asp Val Ala Arg Leu His Leu 165 170 175 | | | | 528 |
| caa ctt gca gca gct cga gtg gcg gca tct tct gaa ggg ctt cat gat Gln Leu Ala Ala Ala Arg Val Ala Ala Ser Ser Glu Gly Leu His Asp 180 185 190 | | | 576 | |
| gtt cat gtg att ttg gta tca gat tgc ttt cca ata ccg aat ctt ttt Val His Val Ile Leu Val Ser Asp Cys Phe Pro Ile Pro Asn Leu Phe 195 200 205 | | | | 624 |
| acg ggt caa gaa ctt gtt gcc cgt caa gga aac ata tgg ctg tat aag Thr Gly Gln Glu Leu Val Ala Arg Gln Gly Asn Ile Trp Leu Tyr Lys 210 215 220 | | | 672 | |
| cct aaa ctt cac cag tta aga caa aag tta caa ctt cct gtt ggt tcc Pro Lys Leu His Gln Leu Arg Gln Lys Leu Gln Leu Pro Val Gly Ser 225 230 235 240 | | | | 720 |
| tgt gaa ctt tct gtt cct ctt caa gct aaa gat-aat ttc tac tcg gca Cys Glu Leu Ser Val Pro Leu Gln Ala Lys Asp Asn Phe Tyr Ser Ala 245 250 255 | | | | 768 |
| aat gcc aag aaa gaa gcg tac gcg acg atc ttg cac tca gat gat gct Asn Ala Lys Lys Glu Ala Tyr Ala Thr Ile Leu His Ser Asp Asp Ala 260 265 270 | | | 816 | |
| ttt gtc tgt gga gcc att gca gta gca cag agc att cga atg tca ggc Phe Val Cys Gly Ala Ile Ala Val Ala Gln Ser Ile Arg Met Ser Gly 275 280 285 | | | | 864 |
| tct act cgc aat ttg gta ata cta gtc gat gat tcg atc agt gaa tac Ser Thr Arg Asn Leu Val Ile Leu Val Asp Asp Ser Ile Ser Glu Tyr 290 295 300 | | | | 912 |
| cat aga agt ggc ttg gaa tca gct gga tgg aag att cac aca ttt caa His Arg Ser Gly Leu Glu Ser Ala Gly Trp Lys Ile His Thr Phe Gln 305 310 315 320 | | | 960 | |

| | |
|---|-------|
| aga atc aga aac ccg aaa gct gaa gca aat gca tat aac caa tgg aac | 1008 |
| Arg Ile Arg Asn Pro Lys Ala Glu Ala Asn Ala Tyr Asn Gln Trp Asn | |
| 325 | 330 |
| 335 | |
| | |
| tac agc aaa ttc cgt ctt tgg gaa ttg aca gaa tac aac aag atc atc | 1056 |
| Tyr Ser Lys Phe Arg Leu Trp Glu Leu Thr Glu Tyr Asn Lys Ile Ile | |
| 340 | 345 |
| 350 | |
| | |
| ttc att gat gca gac atg ctt atc ctc aga aac atg gat ttc ctc ttc | 1104 |
| Phe Ile Asp Ala Asp Met Leu Ile Leu Arg Asn Met Asp Phe Leu Phe | |
| 355 | 360 |
| 365 | |
| | |
| gag tac ccc gaa atc tcc aca act gga aac gac ggt acg ctc ttc aac | 1152. |
| Glu Tyr Pro Glu Ile Ser Thr Thr Gly Asn Asp Gly Thr Leu Phe Asn | |
| 370 | 375 |
| 380 | |
| | |
| tcc ggt cta atg gtg att gaa cca tca aat tca aca ttc cag tta cta | 1200 |
| Ser Gly Leu Met Val Ile Glu Pro Ser Asn Ser Thr Phe Gln Leu Leu | |
| 385 | 390 |
| 395 | 400 |
| | |
| atg gat cac atc aac gat atc aat tcc tac aat gga gga gac caa ggt | 1248 |
| Met Asp His Ile Asn Asp Ile Asn Ser Tyr Asn Gly Gly Asp Gln Gly | |
| 405 | 410 |
| 415 | |
| | |
| tac ctt aac gag ata ttc aca tgg tgg cat cgg att cca aaa cac atg | 1296 |
| Tyr Leu Asn Glu Ile Phe Thr Trp Trp His Arg Ile Pro Lys His Met | |
| 420 | 425 |
| 430 | |
| | |
| aat ttc ttg aag cat ttc tgg gaa gga gac aca cct aag cac agg aaa | 1344 |
| Asn Phe Leu Lys His Phe Trp Glu Gly Asp Thr Pro Lys His Arg Lys | |
| 435 | 440 |
| 445 | |
| | |
| tct aag acg aga cta ttt gga gct gat cct ccg ata ctc tac gtt ctt | 1392 |
| Ser Lys Thr Arg Leu Phe Gly Ala Asp Pro Pro Ile Leu Tyr Val Leu | |
| 450 | 455 |
| 460 | |
| | |
| cat tac cta ggt tac aac aaa cca tgg gta tgc ttc aga gac tac gat | 1440 |
| His Tyr Leu Gly Tyr Asn Lys Pro Trp Val Cys Phe Arg Asp Tyr Asp | |
| 465 | 470 |
| 475 | 480 |
| | |
| tgc aat tgg aat gtc gtt gga tac cat caa ttc gcg agc gat gaa gca | 1488 |
| Cys Asn Trp Asn Val Val Gly Tyr His Gln Phe Ala Ser Asp Glu Ala | |
| 485 | 490 |
| 495 | |
| | |
| cac aaa act tgg tgg aga gtg cac gac gcg atg cct aag aaa ttg cag | 1536 |
| His Lys Thr Trp Trp Arg Val His Asp Ala Met Pro Lys Lys Leu Gln | |
| 500 | 505 |
| 510 | |
| | |
| agg ttt tgt cta ctg agt tcg aaa caa aag gcg caa ctt gag tgg gat | 1584 |
| Arg Phe Cys Leu Leu Ser Ser Lys Gln Lys Ala Gln Leu Glu Trp Asp | |
| 515 | 520 |
| 525 | |

cg_g ag_a ca_a g_ct g_ag a_{aa} g_cg a_{at} t_ac ag_a g_ac g_ga c_at t_gg a_{gg} a_{tt} 1632
 Arg Arg Gln Ala Glu Lys Ala Asn Tyr Arg Asp Gly His Trp Arg Ile
 530 535 540

a_ag a_{tc} a_{aa} g_at a_ag a_ga c_{tt} a_cg a_ct t_gt t_{tt}g g_aa g_at t_{tc} t_gt t_{tc} 1680
 Lys Ile Lys Asp Lys Arg Leu Thr Thr Cys Phe Glu Asp Phe Cys Phe
 545 550 555 560

t_{gg} g_ag a_{gt} a_{tg} c_{tt} t_{gg} c_at t_{gg} g_gc g_at t_{at} g_aa a_{tt} c_{tc} g_aa c_{cc} 1728
 Trp Glu Ser Met Leu Trp His Trp Gly Asp Tyr Glu Ile Leu Glu Thr
 565 570 575

g_ac c_ct g_gt c_{tt} a_cg g_ag a_cg a_{tg} a_{ta} c_ct t_{cc} t_ca a_{gt} c_{cc} a_{tg} g_ag 1776
 Asp Pro Gly Leu Thr Glu Thr Met Ile Pro Ser Ser Ser Pro Met Glu
 580 585 590

t_ca a_ga c_at c_ga c_{tc} t_cg t_{tc} t_ca a_{at} g_ag a_ag a_ca a_{gt} a_{gg} a_{gg} a_{ga} 1824
 Ser Arg His Arg Leu Ser Phe Ser Asn Glu Lys Thr Ser Arg Arg Arg
 595 600 605

t_{tt} c_{aa} a_ga a_{tt} g_ag a_ag g_gt g_{tc} a_ag t_{tc} a_ac a_ct c_{tg} a_{aa} c_{tt} g_{tg} 1872
 Phe Gln Arg Ile Glu Lys Gly Val Lys Phe Asn Thr Leu Lys Leu Val
 610 615 620

t_{tg} a_{tt} t_{gt} a_{ta} a_{tg} c_{tt} g_ga g_ct t_{tg} t_{tc} a_cg a_{tc} t_ac c_{gt} t_{tt} c_{gt} 1920
 Leu Ile Cys Ile Met Leu Gly Ala Leu Phe Thr Ile Tyr Arg Phe Arg
 625 630 635 640

t_{at} c_{ca} c_{cg} c_{ta} c_{aa} a_{tt} c_ct g_aa a_{tt} c_{ca} a_ct a_{gt} t_{tt} g_gt c_{tt} a_ct 1968
 Tyr Pro Pro Leu Gln Ile Pro Glu Ile Pro Thr Ser Phe Gly Leu Thr
 645 650 655

a_ct g_at c_ct c_gc t_{at} g_ta g_ct a_ca g_ct g_ag a_{tc} a_ac t_{gg} a_ac c_at a_{tg} 2016
 Thr Asp Pro Arg Tyr Val Ala Thr Ala Glu Ile Asn Trp Asn His Met
 660 665 670

t_ca a_{at} c_{tt} g_{tt} g_ag a_ag c_ac g_ta t_{tt} g_gt a_ga a_gc g_ag t_{at} c_{aa} g_ga 2064
 Ser Asn Leu Val Glu Lys His Val Phe Gly Arg Ser Glu Tyr Gln Gly
 675 680 685

a_{tt} g_{gt} c_{tt} a_{ta} a_{at} c_{tt} a_ac g_at a_ac g_ag a_{tt} g_at c_{ga} t_{tc} a_ag g_ag 2112
 Ile Gly Leu Ile Asn Leu Asn Asp Asn Glu Ile Asp Arg Phe Lys Glu
 690 695 700

g_ta a_{cg} a_{aa} t_ct g_ac t_gt g_at c_at g_ta g_ct t_{tg} c_at c_{ta} g_at t_{at} g_ct 2160
 Val Thr Lys Ser Asp Cys Asp His Val Ala Leu His Leu Asp Tyr Ala
 705 710 715 720

g_ca a_ag a_ac a_{ta} a_ca t_{gg} g_aa t_ct t_{ta} t_ac c_{cg} g_aa t_{gg} a_{tt} g_at g_aa 2208
 Ala Lys Asn Ile Thr Trp Glu Ser Leu Tyr Pro Glu Trp Ile Asp Glu
 725 730 735

| | |
|---|------|
| gtt gaa gaa ttc gaa gtc cct act tgt cct tct ctg cct ttg att caa | 2256 |
| Val Glu Glu Phe Glu Val Pro Thr Cys Pro Ser Leu Pro Leu Ile Gln | |
| 740 745 750 | |
| att cct ggc aag cct cgg att gat ctt gta att gcc aag ctt ccg tgt | 2304 |
| Ile Pro Gly Lys Pro Arg Ile Asp Leu Val Ile Ala Lys Leu Pro Cys | |
| 755 760 765 | |
| gat aaa tca gga aaa tgg tct aga gat gtg gct cgc ttg cat tta caa | 2352 |
| Asp Lys Ser Gly Lys Trp Ser Arg Asp Val Ala Arg Leu His Leu Gln | |
| 770 775 780 | |
| ctt gca gca gct cga gtg gcg gct tct tct aaa gga ctt cat aat gtt | 2400 |
| Leu Ala Ala Ala Arg Val Ala Ala Ser Ser Lys Gly Leu His Asn Val | |
| 785 790 795 800 | |
| cat gtg att ttg gta tct gat tgc ttt cca ata ccg aat ctt ttt acg | 2448 |
| His Val Ile Leu Val Ser Asp Cys Phe Pro Ile Pro Asn Leu Phe Thr | |
| 805 810 815 | |
| ggt caa gaa ctt gtt gcc cgt caa gga aac ata tgg ctg tat aag cct | 2496 |
| Gly Gln Glu Leu Val Ala Arg Gln Gly Asn Ile Trp Leu Tyr Lys Pro | |
| 820 825 830 | |
| aat ctt cac cag cta aga caa aag tta cag ctt cct gtt ggt tcc tgt | 2544 |
| Asn Leu His Gln Leu Arg Gln Lys Leu Gln Leu Pro Val Gly Ser Cys | |
| 835 840 845 | |
| gaa ctt tct gtt cct ctt caa gct aaa gat aat ttc tac tcc gca ggt | 2592 |
| Glu Leu Ser Val Pro Leu Gln Ala Lys Asp Asn Phe Tyr Ser Ala Gly | |
| 850 855 860 | |
| gca aag aaa gaa gct tac gcg act atc ttg cat tct gcc caa ttt tat | 2640 |
| Ala Lys Lys Glu Ala Tyr Ala Thr Ile Leu His Ser Ala Gln Phe Tyr | |
| 865 870 875 880 | |
| gtc tgt gga gcc att gca gct gca cag agc att cga atg tca ggc tct | 2688 |
| Val Cys Gly Ala Ile Ala Ala Gln Ser Ile Arg Met Ser Gly Ser | |
| 885 890 895 | |
| act cgt gat ctg gtc ata ctt gtt gat gaa acg ata agc gaa tac cat | 2736 |
| Thr Arg Asp Leu Val Ile Leu Val Asp Glu Thr Ile Ser Glu Tyr His | |
| 900 905 910 | |
| aaa agt ggc ttg gta gct ggt gga tgg aag att caa atg ttt caa aga | 2784 |
| Lys Ser Gly Leu Val Ala Ala Gly Trp Lys Ile Gln Met Phe Gln Arg | |
| 915 920 925 | |
| atc agg aac ccg aat gct gta cca aat gcc tac aac gaa tgg aac tac | 2832 |
| Ile Arg Asn Pro Asn Ala Val Pro Asn Ala Tyr Asn Glu Trp Asn Tyr | |
| 930 935 940 | |

| | |
|---|------|
| agc aag ttt cgt ctt tgg caa ctg act gaa tac agt aag atc atc ttc Ser Lys Phe Arg Leu Trp Gln Leu Thr Glu Tyr Ser Lys Ile Ile Phe 945 950 955 960 | 2880 |
| atc gat gca gac atg ctt atc ctg aga aac att gat ttc ctc ttc gag Ile Asp Ala Asp Met Leu Ile Leu Arg Asn Ile Asp Phe Leu Phe Glu 965 970 975 | 2928 |
| ttc cct gag ata tca gca act gga aac aat gct acg ctc ttc aac tct Phe Pro Glu Ile Ser Ala Thr Gly Asn Asn Ala Thr Leu Phe Asn Ser 980 985 990 | 2976 |
| ggt cta atg gtg gtt gag cca tct aat tca aca ttc cag tta cta atg Gly Leu Met Val Val Glu Pro Ser Asn Ser Thr Phe Gln Leu Leu Met 995 1000 1005 | 3024 |
| gat aac att aat gaa gtt gtg tct tac aac gga gga gac caa ggt tac Asp Asn Ile Asn Glu Val Val Ser Tyr Asn Gly Gly Asp Gln Gly Tyr 1010 1015 1020 | 3072 |
| ctt aac gag ata ttc aca tgg tgg cat cgg att cca aaa cac atg aat Leu Asn Glu Ile Phe Thr Trp Trp His Arg Ile Pro Lys His Met Asn 1025 1030 1035 1040 | 3120 |
| ttc ttg aag cat ttc tgg gaa gga gac gaa cct gag att aaa aaa atg Phe Leu Lys His Phe Trp Glu Gly Asp Glu Pro Glu Ile Lys Lys Met 1045 1050 1055 | 3168 |
| aag acg agt cta ttt gga gct gat cct ccg atc cta tac gtt ctt cat Lys Thr Ser Leu Phe Gly Ala Asp Pro Pro Ile Leu Tyr Val Leu His 1060 1065 1070 | 3216 |
| tac cta ggt tat aac aaa ccc tgg tta tgc ttc aga gac tat gac tgc Tyr Leu Gly Tyr Asn Lys Pro Trp Leu Cys Phe Arg Asp Tyr Asp Cys 1075 1080 1085 | 3264 |
| aat tgg aat gtc gat att ttc cag gaa ttt gct agt gac gag gct cat Asn Trp Asn Val Asp Ile Phe Gln Glu Phe Ala Ser Asp Glu Ala His 1090 1095 1100 | 3312 |
| aaa acc tgg tgg aga gtg cac gac gca atg cct gaa aac ttg cat aag Lys Thr Trp Trp Arg Val His Asp Ala Met Pro Glu Asn Leu His Lys 1105 1110 1115 1120 | 3360 |
| ttc tgt cta cta aga tcg aaa cag aag gcg caa ctt gaa tgg gat agg Phe Cys Leu Leu Arg Ser Lys Gln Lys Ala Gln Leu Glu Trp Asp Arg 1125 1130 1135 | 3408 |
| aga caa gca gag aaa ggg aac tac aaa gat gga cat tgg aag ata aag Arg Gln Ala Glu Lys Gly Asn Tyr Lys Asp Gly His Trp Lys Ile Lys 1140 1145 1150 | 3456 |

atc aaa gac aag aga ctt aag act tgt ttc gaa gat ttc tgc ttt tgg 3504
 Ile Lys Asp Lys Arg Leu Lys Thr Cys Phe Glu Asp Phe Cys Phe Trp
 1155 1160 1165

gag agt atg ctt tgg cat tgg ggt gag acg aac tct acc aac aat tct 3552
 Glu Ser Met Leu Trp His Trp Gly Glu Thr Asn Ser Thr Asn Asn Ser
 1170 1175 1180

tcc acc acc acc act tca tca ccg ccg cat aaa acc gct ctc cct tcc 3600
 Ser Thr Thr Thr Ser Ser Pro Pro His Lys Thr Ala Leu Pro Ser
 1185 1190 1195 1200

ctg tga 3606
 Leu

<210> 7
 <211> 1201
 <212> PRT
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<400> 7
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 1 5 10 15
 Leu Val Ala Met Phe Thr Leu Tyr Cys Ser Pro Pro Leu Gln Ile Pro
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 Glu Asp Pro Ser Ser Phe Ala Asn Lys Trp Ile Leu Glu Pro Ala Val
 35 40 45
 Thr Thr Asp Pro Arg Tyr Ile Ala Thr Ser Glu Ile Asn Trp Asn Ser
 50 55 60
 Met Ser Leu Val Val Glu His Tyr Leu Ser Gly Arg Ser Glu Tyr Gln
 65 70 75 80
 Gly Ile Gly Phe Leu Asn Leu Asp Asn Glu Ile Asn Arg Trp Gln
 85 90 95
 Val Val Ile Lys Ser His Cys Gln His Ile Ala Leu His Leu Asp His
 100 105 110
 Ala Ala Ser Asn Ile Thr Trp Lys Ser Leu Tyr Pro Glu Trp Ile Asp
 115 120 125
 Glu Glu Glu Lys Phe Lys Val Pro Thr Cys Pro Ser Leu Pro Trp Ile
 130 135 140
 Gln Val Pro Asp Lys Ser Arg Ile Asp Leu Ile Ile Ala Lys Leu Pro
 145 150 155 160
 Cys Asn Lys Ser Gly Lys Trp Ser Arg Asp Val Ala Arg Leu His Leu
 165 170 175
 Gln Leu Ala Ala Ala Arg Val Ala Ala Ser Ser Glu Gly Leu His Asp
 180 185 190
 Val His Val Ile Leu Val Ser Asp Cys Phe Pro Ile Pro Asn Leu Phe
 195 200 205
 Thr Gly Gln Glu Leu Val Ala Arg Gln Gly Asn Ile Trp Leu Tyr Lys
 210 215 220
 Pro Lys Leu His Gln Leu Arg Gln Lys Leu Gln Leu Pro Val Gly Ser

16

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 225 | 230 | 235 | 240 | | | | | | | | | | | | |
| Cys | Glu | Leu | Ser | Val | Pro | Leu | Gln | Ala | Lys | Asp | Asn | Phe | Tyr | Ser | Ala |
| 245 | | | | | | | | | 250 | | | | | | 255 |
| Asn | Ala | Lys | Lys | Glu | Ala | Tyr | Ala | Thr | Ile | Leu | His | Ser | Asp | Asp | Ala |
| 260 | | | | | | | | 265 | | | | | | 270 | |
| Phe | Val | Cys | Gly | Ala | Ile | Ala | Val | Ala | Gln | Ser | Ile | Arg | Met | Ser | Gly |
| 275 | | | | | | | 280 | | | | | 285 | | | |
| Ser | Thr | Arg | Asn | Leu | Val | Ile | Leu | Val | Asp | Asp | Ser | Ile | Ser | Glu | Tyr |
| 290 | | | | | | 295 | | | | | 300 | | | | |
| His | Arg | Ser | Gly | Leu | Glu | Ser | Ala | Gly | Trp | Lys | Ile | His | Thr | Phe | Gln |
| 305 | | | | 310 | | | | | 315 | | | | | 320 | |
| Arg | Ile | Arg | Asn | Pro | Lys | Ala | Glu | Ala | Asn | Ala | Tyr | Asn | Gln | Trp | Asn |
| 325 | | | | | | | | 330 | | | | | | 335 | |
| Tyr | Ser | Lys | Phe | Arg | Leu | Trp | Glu | Leu | Thr | Glu | Tyr | Asn | Lys | Ile | Ile |
| 340 | | | | | | | 345 | | | | | 350 | | | |
| Phe | Ile | Asp | Ala | Asp | Met | Leu | Ile | Leu | Arg | Asn | Met | Asp | Phe | Leu | Phe |
| 355 | | | | | | | 360 | | | | | 365 | | | |
| Glu | Tyr | Pro | Glu | Ile | Ser | Thr | Thr | Gly | Asn | Asp | Gly | Thr | Leu | Phe | Asn |
| 370 | | | | | | 375 | | | | | 380 | | | | |
| Ser | Gly | Leu | Met | Val | Ile | Glu | Pro | Ser | Asn | Ser | Thr | Phe | Gln | Leu | Leu |
| 385 | | | | | | 390 | | | | 395 | | | | 400 | |
| Met | Asp | His | Ile | Asn | Asp | Ile | Asn | Ser | Tyr | Asn | Gly | Gly | Asp | Gln | Gly |
| 405 | | | | | | | | | 410 | | | | | 415 | |
| Tyr | Leu | Asn | Glu | Ile | Phe | Thr | Trp | Trp | His | Arg | Ile | Pro | Lys | His | Met |
| 420 | | | | | | | | 425 | | | | | 430 | | |
| Asn | Phe | Leu | Lys | His | Phe | Trp | Glu | Gly | Asp | Thr | Pro | Lys | His | Arg | Lys |
| 435 | | | | | | | 440 | | | | | 445 | | | |
| Ser | Lys | Thr | Arg | Leu | Phe | Gly | Ala | Asp | Pro | Pro | Ile | Leu | Tyr | Val | Leu |
| 450 | | | | | | | 455 | | | | | 460 | | | |
| His | Tyr | Leu | Gly | Tyr | Asn | Lys | Pro | Trp | Val | Cys | Phe | Arg | Asp | Tyr | Asp |
| 465 | | | | | | | 470 | | | | 475 | | | | 480 |
| Cys | Asn | Trp | Asn | Val | Val | Gly | Tyr | His | Gln | Phe | Ala | Ser | Asp | Glu | Ala |
| 485 | | | | | | | | 490 | | | | | | 495 | |
| His | Lys | Thr | Trp | Trp | Arg | Val | His | Asp | Ala | Met | Pro | Lys | Lys | Leu | Gln |
| 500 | | | | | | | | 505 | | | | | | 510 | |
| Arg | Phe | Cys | Leu | Leu | Ser | Ser | Lys | Gln | Lys | Ala | Gln | Leu | Glu | Trp | Asp |
| 515 | | | | | | | | 520 | | | | | | 525 | |
| Arg | Arg | Gln | Ala | Glu | Lys | Ala | Asn | Tyr | Arg | Asp | Gly | His | Trp | Arg | Ile |
| 530 | | | | | | | | 535 | | | | | 540 | | |
| Lys | Ile | Lys | Asp | Lys | Arg | Leu | Thr | Thr | Cys | Phe | Glu | Asp | Phe | Cys | Phe |
| 545 | | | | | | | | 550 | | | | 555 | | | 560 |
| Trp | Glu | Ser | Met | Leu | Trp | His | Trp | Gly | Asp | Tyr | Glu | Ile | Leu | Glu | Thr |
| 565 | | | | | | | | 570 | | | | | | 575 | |
| Asp | Pro | Gly | Leu | Thr | Glu | Thr | Met | Ile | Pro | Ser | Ser | Ser | Pro | Met | Glu |
| 580 | | | | | | | | 585 | | | | | | 590 | |
| Ser | Arg | His | Arg | Leu | Ser | Phe | Ser | Asn | Glu | Lys | Thr | Ser | Arg | Arg | Arg |
| 595 | | | | | | | | 600 | | | | | | 605 | |
| Phe | Gln | Arg | Ile | Glu | Lys | Gly | Val | Lys | Phe | Asn | Thr | Leu | Lys | Leu | Val |
| 610 | | | | | | | | 615 | | | | | 620 | | |
| Leu | Ile | Cys | Ile | Met | Leu | Gly | Ala | Leu | Phe | Thr | Ile | Tyr | Arg | Phe | Arg |
| 625 | | | | | | | | 630 | | | | 635 | | | 640 |
| Tyr | Pro | Pro | Leu | Gln | Ile | Pro | Glu | Ile | Pro | Thr | Ser | Phe | Gly | Leu | Thr |

| 645 | 650 | 655 |
|---|------|------|
| Thr Asp Pro Arg Tyr Val Ala Thr Ala Glu Ile Asn Trp Asn His Met | | |
| 660 | 665 | 670 |
| Ser Asn Leu Val Glu Lys His Val Phe Gly Arg Ser Glu Tyr Gln Gly | | |
| 675 | 680 | 685 |
| Ile Gly Leu Ile Asn Leu Asn Asp Asn Glu Ile Asp Arg Phe Lys Glu | | |
| 690 | 695 | 700 |
| Val Thr Lys Ser Asp Cys Asp His Val Ala Leu His Leu Asp Tyr Ala | | |
| 705 | 710 | 715 |
| Ala Lys Asn Ile Thr Trp Glu Ser Leu Tyr Pro Glu Trp Ile Asp Glu | | |
| 725 | 730 | 735 |
| Val Glu Glu Phe Glu Val Pro Thr Cys Pro Ser Leu Pro Leu Ile Gln | | |
| 740 | 745 | 750 |
| Ile Pro Gly Lys Pro Arg Ile Asp Leu Val Ile Ala Lys Leu Pro Cys | | |
| 755 | 760 | 765 |
| Asp Lys Ser Gly Lys Trp Ser Arg Asp Val Ala Arg Leu His Leu Gln | | |
| 770 | 775 | 780 |
| Leu Ala Ala Ala Arg Val Ala Ala Ser Ser Lys Gly Leu His Asn Val | | |
| 785 | 790 | 795 |
| His Val Ile Leu Val Ser Asp Cys Phe Pro Ile Pro Asn Leu Phe Thr | | |
| 805 | 810 | 815 |
| Gly Gln Glu Leu Val Ala Arg Gln Gly Asn Ile Trp Leu Tyr Lys Pro | | |
| 820 | 825 | 830 |
| Asn Leu His Gln Leu Arg Gln Lys Leu Gln Leu Pro Val Gly Ser Cys | | |
| 835 | 840 | 845 |
| Glu Leu Ser Val Pro Leu Gln Ala Lys Asp Asn Phe Tyr Ser Ala Gly | | |
| 850 | 855 | 860 |
| Ala Lys Lys Glu Ala Tyr Ala Thr Ile Leu His Ser Ala Gln Phe Tyr | | |
| 865 | 870 | 875 |
| Val Cys Gly Ala Ile Ala Ala Ala Gln Ser Ile Arg Met Ser Gly Ser | | |
| 885 | 890 | 895 |
| Thr Arg Asp Leu Val Ile Leu Val Asp Glu Thr Ile Ser Glu Tyr His | | |
| 900 | 905 | 910 |
| Lys Ser Gly Leu Val Ala Ala Gly Trp Lys Ile Gln Met Phe Gln Arg | | |
| 915 | 920 | 925 |
| Ile Arg Asn Pro Asn Ala Val Pro Asn Ala Tyr Asn Glu Trp Asn Tyr | | |
| 930 | 935 | 940 |
| Ser Lys Phe Arg Leu Trp Gln Leu Thr Glu Tyr Ser Lys Ile Ile Phe | | |
| 945 | 950 | 955 |
| Ile Asp Ala Asp Met Leu Ile Leu Arg Asn Ile Asp Phe Leu Phe Glu | | |
| 965 | 970 | 975 |
| Phe Pro Glu Ile Ser Ala Thr Gly Asn Asn Ala Thr Leu Phe Asn Ser | | |
| 980 | 985 | 990 |
| Gly Leu Met Val Val Glu Pro Ser Asn Ser Thr Phe Gln Leu Leu Met | | |
| 995 | 1000 | 1005 |
| Asp Asn Ile Asn Glu Val Val Ser Tyr Asn Gly Gly Asp Gln Gly Tyr | | |
| 1010 | 1015 | 1020 |
| Leu Asn Glu Ile Phe Thr Trp Trp His Arg Ile Pro Lys His Met Asn | | |
| 1025 | 1030 | 1035 |
| Phe Leu Lys His Phe Trp Glu Gly Asp Glu Pro Glu Ile Lys Lys Met | | |
| 1045 | 1050 | 1055 |
| Lys Thr Ser Leu Phe Gly Ala Asp Pro Pro Ile Leu Tyr Val Leu His | | |

18

| | | |
|---|------|------|
| 1060 | 1065 | 1070 |
| Tyr Leu Gly Tyr Asn Lys Pro Trp Leu Cys Phe Arg Asp Tyr Asp Cys | | |
| 1075 | 1080 | 1085 |
| Asn Trp Asn Val Asp Ile Phe Gln Glu Phe Ala Ser Asp Glu Ala His | | |
| 1090 | 1095 | 1100 |
| Lys Thr Trp Trp Arg Val His Asp Ala Met Pro Glu Asn Leu His Lys | | |
| 1105 | 1110 | 1115 |
| Phe Cys Leu Leu Arg Ser Lys Gln Lys Ala Gln Leu Glu Trp Asp Arg | | |
| 1125 | 1130 | 1135 |
| Arg Gln Ala Glu Lys Gly Asn Tyr Lys Asp Gly His Trp Lys Ile Lys | | |
| 1140 | 1145 | 1150 |
| Ile Lys Asp Lys Arg Leu Lys Thr Cys Phe Glu Asp Phe Cys Phe Trp | | |
| 1155 | 1160 | 1165 |
| Glu Ser Met Leu Trp His Trp Gly Glu Thr Asn Ser Thr Asn Asn Ser | | |
| 1170 | 1175 | 1180 |
| Ser Thr Thr Thr Ser Ser Pro Pro His Lys Thr Ala Leu Pro Ser | | |
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| Leu | | 1200 |

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| atg | ggg | gcc | aaa | agc | aaa | agt | tcg | agt | acg | aga | ttt | ttt | atg | ttt | tat | 48 |
| Met | Gly | Ala | Lys | Ser | Lys | Ser | Ser | Ser | Thr | Arg | Phe | Phe | Met | Phe | Tyr | |
| 1 | | 5 | | | | | | | 10 | | | | 15 | | | |
| ctt ata cta ata tca ttg tcg ttt ttg ggt ttg ctc tta aac ttt aaa | | | | | | | | | | | | | | | | 96 |
| Leu | Ile | Leu | Ile | Ser | Leu | Ser | Phe | Leu | Gly | Leu | Leu | Leu | Asn | Phe | Lys | |
| | | | | | | | | 20 | | 25 | | | 30 | | | |
| cct ctg ttt ctg ctc aac ccc atg atc gct tct cct tcg ata gtt gag | | | | | | | | | | | | | | | | 144 |
| Pro | Leu | Phe | Leu | Leu | Asn | Pro | Met | Ile | Ala | Ser | Pro | Ser | Ile | Val | Glu | |
| | 35 | | 40 | | | | | | | | | | 45 | | | |
| att cgt tat tct ttg ccg gaa ccg gtt aaa ccg act ccg ata tgg ctc | | | | | | | | | | | | | | | | 192 |
| Ile | Arg | Tyr | Ser | Leu | Pro | Glu | Pro | Val | Lys | Arg | Thr | Pro | Ile | Trp | Leu | |
| | 50 | | 55 | | | | | | 60 | | | | | | | |
| cga ctc att aga aac tat ctt ccg gat gag aaa aag atc cga gtg ggt | | | | | | | | | | | | | | | | 240 |
| Arg | Leu | Ile | Arg | Asn | Tyr | Leu | Pro | Asp | Glu | Lys | Lys | Ile | Arg | Val | Gly | |
| | 65 | | 70 | | | | | | 75 | | | | 80 | | | |
| ctt ctc aac atc gca gag aac gag cga gag agc tac gag gca agc ggg | | | | | | | | | | | | | | | | 288 |

19

Leu Leu Asn Ile Ala Glu Asn Glu Arg Glu Ser Tyr Glu Ala Ser Gly
 85 90 95

acg tcg atc ttg gag aat gtc cac gtg tcg ctc gat cct ctt ccg aac 336
 Thr Ser Ile Leu Glu Asn Val His Val Ser Leu Asp Pro Leu Pro Asn
 100 105 110

aat ctg aca tgg acg agt tta ttc ccg gtt tgg atc gac gag gat cac 384
 Asn Leu Thr Trp Thr Ser Leu Phe Pro Val Trp Ile Asp Glu Asp His
 115 120 125

acg tgg cac att cct agt tgt cca gaa gtc cct ctc cct aag atg gaa 432
 Thr Trp His Ile Pro Ser Cys Pro Glu Val Pro Leu Pro Lys Met Glu
 130 135 140

ggg tcc gaa gct gac gtg gac gtc gtc gtt gtc aaa gtc ccg tgc gat 480
 Gly Ser Glu Ala Asp Val Asp Val Val Val Lys Val Pro Cys Asp
 145 150 155 160

ggg ttc tcg gag aag aga ggg tta aga gac gtt ttc agg cta cag gtg 528
 Gly Phe Ser Glu Lys Arg Gly Leu Arg Asp Val Phe Arg Leu Gln Val
 165 170 175

aat ctg gcg gca gcg aat ctt gtg gtg gag agt ggt cgg agg aat gtt 576
 Asn Leu Ala Ala Ala Asn Leu Val Val Glu Ser Gly Arg Arg Asn Val
 180 185 190

gat cgg act gtg tac gtt gtc ttc atc gga tct tgt ggg cct atg cat 624
 Asp Arg Thr Val Tyr Val Val Phe Ile Gly Ser Cys Gly Pro Met His
 195 200 205

gag atc ttt agg tgt gat gag cgc gtg aag cgc gtg ggg gac tat tgg 672
 Glu Ile Phe Arg Cys Asp Glu Arg Val Lys Arg Val Gly Asp Tyr Trp
 210 215 220

gtc tat agg cct gat ctt acg agg ttg aag cag aag ctt ctc atg cct 720
 Val Tyr Arg Pro Asp Leu Thr Arg Leu Lys Gln Lys Leu Leu Met Pro
 225 230 235 240

cct ggt tca tgt cag att gct ccg cta ggt caa gga gaa gca tgg ata 768
 Pro Gly Ser Cys Gln Ile Ala Pro Leu Gly Gln Gly Glu Ala Trp Ile
 245 250 255

caa gac aag aac aga aat ctc aca tcc gaa aaa act aca tta tca tca 816
 Gln Asp Lys Asn Arg Asn Leu Thr Ser Glu Lys Thr Thr Leu Ser Ser
 260 265 270

ttt act gcc caa cgt gtc gct tac gtg acg tta cta cac tca tcg gag 864
 Phe Thr Ala Gln Arg Val Ala Tyr Val Thr Leu Leu His Ser Ser Glu
 275 280 285

gta tac gta tgc gga gca ata gcc tta gca caa agc ata agg caa tct 912

20

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|------|
| Val | Tyr | Val | Cys | Gly | Ala | Ile | Ala | Leu | Ala | Gln | Ser | Ile | Arg | Gln | Ser | 290 | 295 | 300 | 960 | | | | | | | | | | | | | | | | | |
| gga | tca | acc | aag | gac | atg | att | ctc | ctc | cac | gat | gac | tct | ata | acc | aac | Gly | Ser | Thr | Lys | Asp | Met | Ile | Leu | Leu | His | Asp | Asp | Ser | Ile | Thr | Asn | 305 | 310 | 315 | 320 | |
| atc | tct | ctc | att | ggc | cta | agc | ctt | gct | ggc | tgg | aaa | cta | cgg | cga | gtg | Ile | Ser | Leu | Ile | Gly | Leu | Ser | Leu | Ala | Gly | Trp | Lys | Leu | Arg | Arg | Val | 325 | 330 | 335 | 1008 | |
| gag | aga | att | cgt | agt | cct | ttt | tcc | aag | aag | cgt | tct | tac | aat | gag | tgg | Glu | Arg | Ile | Arg | Ser | Pro | Phe | Ser | Lys | Lys | Arg | Ser | Tyr | Asn | Glu | Trp | 340 | 345 | 350 | 1056 | |
| aac | tac | agt | aag | tta | cgt | gtg | tgg | caa | gtg | aca | gat | tac | gac | aaa | cta | Asn | Tyr | Ser | Lys | Leu | Arg | Val | Trp | Gln | Val | Thr | Asp | Tyr | Asp | Lys | Leu | 355 | 360 | 365 | 1104 | |
| gtg | ttt | ata | gac | gca | gac | ttc | atc | atc | gtc | aag | aat | att | gat | tac | ctt | Val | Phe | Ile | Asp | Ala | Asp | Phe | Ile | Ile | Val | Lys | Asn | Ile | Asp | Tyr | Leu | 370 | 375 | 380 | 1152 | |
| ttc | tcc | tat | cct | caa | ctt | tct | gcc | gct | ggc | aat | aac | aaa | gtc | ttg | ttc | Phe | Ser | Tyr | Pro | Gln | Leu | Ser | Ala | Ala | Gly | Asn | Asn | Lys | Val | Leu | Phe | 385 | 390 | 395 | 400 | 1200 |
| aac | tca | gga | gtc | atg | gtt | ctg | gag | cca | tca | gct | tgt | tta | ttc | gag | gat | Asn | Ser | Gly | Val | Met | Val | Leu | Glu | Pro | Ser | Ala | Cys | Leu | Phe | Glu | Asp | 405 | 410 | 415 | 1248 | |
| ttg | atg | ctt | aaa | tca | ttc | aag | atc | ggg | tca | tac | aac | ggg | gga | gac | caa | Leu | Met | Leu | Lys | Ser | Phe | Lys | Ile | Gly | Ser | Tyr | Asn | Gly | Gly | Asp | Gln | 420 | 425 | 430 | 1296 | |
| gga | ttt | ctg | aac | gaa | tat | ttc | gtg | tgg | tgg | cat | agg | cat | gat | aaa | gcg | Gly | Phe | Leu | Asn | Glu | Tyr | Phe | Val | Trp | Trp | His | Arg | His | Asp | Lys | Ala | 435 | 440 | 445 | 1344 | |
| cgc | aat | ctt | cca | gaa | aat | tta | gag | ggc | ata | cac | tac | ttg | gga | cta | aaa | Arg | Asn | Leu | Pro | Glu | Asn | Leu | Glu | Gly | Ile | His | Tyr | Leu | Gly | Leu | Lys | 450 | 455 | 460 | 1392 | |
| cca | tgg | cga | tgt | aga | gac | tac | gat | tgt | aac | tgg | gac | ttg | aaa | acg | 465 | 470 | 475 | 480 | Pro | Trp | Arg | Cys | Tyr | Arg | Asp | Tyr | Asp | Cys | Asn | Trp | Asp | Leu | Lys | Thr | | |
| cga | cgt | gtg | tat | gca | agc | gag | tcg | gtg | cat | gcg | aga | tgg | tgg | aaa | gtg | Arg | Arg | Val | Tyr | Ala | Ser | Glu | Ser | Val | His | Ala | Arg | Trp | Trp | Lys | Val | 485 | 490 | 495 | 1488 | |
| tac | gac | aag | atg | cct | aag | aag | ctg | aaa | ggt | tat | tgt | ggt | ttg | aat | ctt | 1536 | | | | | | | | | | | | | | | | | | | | |

21

Tyr Asp Lys Met Pro Lys Lys Leu Lys Gly Tyr Cys Gly Leu Asn Leu
 500 505 510

aag atg gag aag aac gtt gag aag tgg agg aaa atg gct aag ctc aat 1584
 Lys Met Glu Lys Asn Val Glu Lys Trp Arg Lys Met Ala Lys Leu Asn
 515 520 525

ggt ttt cct gaa aat cat tgg aaa att aga ata aaa gat cct agg aag 1632
 Gly Phe Pro Glu Asn His Trp Lys Ile Arg Ile Lys Asp Pro Arg Lys
 530 535 540

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 Pro Leu Phe Leu Leu Asn Pro Met Ile Ala Ser Pro Ser Ile Val Glu
 35 40 45
 Ile Arg Tyr Ser Leu Pro Glu Pro Val Lys Arg Thr Pro Ile Trp Leu
 50 55 60
 Arg Leu Ile Arg Asn Tyr Leu Pro Asp Glu Lys Ile Arg Val Gly
 65 70 75 80
 Leu Leu Asn Ile Ala Glu Asn Glu Arg Glu Ser Tyr Glu Ala Ser Gly
 85 90 95
 Thr Ser Ile Leu Glu Asn Val His Val Ser Leu Asp Pro Leu Pro Asn
 100 105 110
 Asn Leu Thr Trp Thr Ser Leu Phe Pro Val Trp Ile Asp Glu Asp His
 115 120 125
 Thr Trp His Ile Pro Ser Cys Pro Glu Val Pro Leu Pro Lys Met Glu
 130 135 140
 Gly Ser Glu Ala Asp Val Asp Val Val Val Lys Val Pro Cys Asp
 145 150 155 160
 Gly Phe Ser Glu Lys Arg Gly Leu Arg Asp Val Phe Arg Leu Gln Val
 165 170 175
 Asn Leu Ala Ala Ala Asn Leu Val Val Glu Ser Gly Arg Arg Asn Val
 180 185 190
 Asp Arg Thr Val Tyr Val Val Phe Ile Gly Ser Cys Gly Pro Met His
 195 200 205
 Glu Ile Phe Arg Cys Asp Glu Arg Val Lys Arg Val Gly Asp Tyr Trp
 210 215 220
 Val Tyr Arg Pro Asp Leu Thr Arg Leu Lys Gln Lys Leu Leu Met Pro
 225 230 235 240

22

Pro Gly Ser Cys Gln Ile Ala Pro Leu Gly Gln Gly Glu Ala Trp Ile
 245 250 255
 Gln Asp Lys Asn Arg Asn Leu Thr Ser Glu Lys Thr Thr Leu Ser Ser
 260 265 270
 Phe Thr Ala Gln Arg Val Ala Tyr Val Thr Leu Leu His Ser Ser Glu
 275 280 285
 Val Tyr Val Cys Gly Ala Ile Ala Leu Ala Gln Ser Ile Arg Gln Ser
 290 295 300
 Gly Ser Thr Lys Asp Met Ile Leu Leu His Asp Asp Ser Ile Thr Asn
 305 310 315 320
 Ile Ser Leu Ile Gly Leu Ser Leu Ala Gly Trp Lys Leu Arg Arg Val
 325 330 335
 Glu Arg Ile Arg Ser Pro Phe Ser Lys Lys Arg Ser Tyr Asn Glu Trp
 340 345 350
 Asn Tyr Ser Lys Leu Arg Val Trp Gln Val Thr Asp Tyr Asp Lys Leu
 355 360 365
 Val Phe Ile Asp Ala Asp Phe Ile Ile Val Lys Asn Ile Asp Tyr Leu
 370 375 380
 Phe Ser Tyr Pro Gln Leu Ser Ala Ala Gly Asn Asn Lys Val Leu Phe
 385 390 395 400
 Asn Ser Gly Val Met Val Leu Glu Pro Ser Ala Cys Leu Phe Glu Asp
 405 410 415
 Leu Met Leu Lys Ser Phe Lys Ile Gly Ser Tyr Asn Gly Gly Asp Gln
 420 425 430
 Gly Phe Leu Asn Glu Tyr Phe Val Trp Trp His Arg His Asp Lys Ala
 435 440 445
 Arg Asn Leu Pro Glu Asn Leu Glu Gly Ile His Tyr Leu Gly Leu Lys
 450 455 460
 Pro Trp Arg Cys Tyr Arg Asp Tyr Asp Cys Asn Trp Asp Leu Lys Thr
 465 470 475 480
 Arg Arg Val Tyr Ala Ser Glu Ser Val His Ala Arg Trp Trp Lys Val
 485 490 495
 Tyr Asp Lys Met Pro Lys Lys Leu Lys Gly Tyr Cys Gly Leu Asn Leu
 500 505 510
 Lys Met Glu Lys Asn Val Glu Lys Trp Arg Lys Met Ala Lys Leu Asn
 515 520 525
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 Lys Asn Arg Leu Ser Gln
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cta atc cta gtc tca ttg tca ctt cta ggt ttg atc tta cct ttt aaa 96
Leu Ile Leu Val Ser Leu Ser Leu Leu Gly Leu Ile Leu Pro Phe Lys
20 25 30

cct ctt ttc cgg att act tct cca tct tca acg tta cgg att gat ctt 144
Pro Leu Phe Arg Ile Thr Ser Pro Ser Ser Thr Leu Arg Ile Asp Leu
35 40 45

cca tcg ccg caa gtc aac aaa aac ccg aaa tgg ctt cga ctc atc cgt 192
Pro Ser Pro Gln Val Asn Lys Asn Pro Lys Trp Leu Arg Leu Ile Arg
50 55 60

aac tat cta cca gag aaa aga atc caa gtc ggc ttc ctt aac ata gac 240
Asn Tyr Leu Pro Glu Lys Arg Ile Gln Val Gly Phe Leu Asn Ile Asp
65 70 75 80

gag aaa gag cgt gag agc tac gag gct cgt gga ccg ttg gta ctt aag 288
Glu Lys Glu Arg Glu Ser Tyr Glu Ala Arg Gly Pro Leu Val Leu Lys
85 90 95

aac atc cac gtg ccg ctt gat cat ata ccc aag aat gtc act tgg aag 336
Asn Ile His Val Pro Leu Asp His Ile Pro Lys Asn Val Thr Trp Lys
100 105 110

agt ctt tac ccg gag tgg atc aac gag gaa gct tct acc tgt ccg gag 384
Ser Leu Tyr Pro Glu Trp Ile Asn Glu Glu Ala Ser Thr Cys Pro Glu
115 120 125

atc cct ctc cct cag cca gaa ggt tct gat gct aac gtg gac gtt att 432
Ile Pro Leu Pro Gln Pro Glu Gly Ser Asp Ala Asn Val Asp Val Ile
130 135 140

gtt gct aga gtt cca tgt gat ggt tgg tcg gcg aat aaa ggg ctt agg 480
Val Ala Arg Val Pro Cys Asp Gly Trp Ser Ala Asn Lys Gly Leu Arg
145 150 155 160

gac gtt ttt agg ctt cag gtt aat ttg gcc gca gcg aat cta gcc gtc 528
Asp Val Phe Arg Leu Gln Val Asn Leu Ala Ala Asn Leu Ala Val
165 170 175

caa agt ggg ttg agg acg gtt aat cag gcg gtc tac gtt gta ttc atc 576
Gln Ser Gly Leu Arg Thr Val Asn Gln Ala Val Tyr Val Val Phe Ile
180 185 190

ggc tca tgt ggg cct atg cat gag att ttc ccg tgc gat gag cgc gtc 624
Gly Ser Cys Gly Pro Met His Glu Ile Phe Pro Cys Asp Glu Arg Val
195 200 205

| | |
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| atg cgc gtg gag gat tat tgg gtg tat aag cct tat ctc cca agg ttg | 672 |
| Met Arg Val Glu Asp Tyr Trp Val Tyr Lys Pro Tyr Leu Pro Arg Leu | |
| 210 215 220 | |
| aag cag aag ctt ctc atg cct gtt tca tgt cag att gct cct tca | 720 |
| Lys Gln Lys Leu Leu Met Pro Val Gly Ser Cys Gln Ile Ala Pro Ser | |
| 225 230 235 240 | |
| ttt gct caa ttt ggt caa gaa gca tgg aga cca aaa cat gaa gat aat | 768 |
| Phe Ala Gln Phe Gly Gln Glu Ala Trp Arg Pro Lys His Glu Asp Asn | |
| 245 250 255 | |
| ctt gca tca aag gca gtc aca gcc tta ccc cgt cgc tta cggtt gcc | 816 |
| Leu Ala Ser Lys Ala Val Thr Ala Leu Pro Arg Arg Leu Arg Val Ala | |
| 260 265 270 | |
| tac gtg aca gta cta cac tcg tca gaa gcc tat gtt tgt ggg gca ata | 864 |
| Tyr Val Thr Val Leu His Ser Ser Glu Ala Tyr Val Cys Gly Ala Ile | |
| 275 280 285 | |
| gct tta gcg caa agt ata aga caa tca gga tcg cat aag gac atg att | 912 |
| Ala Leu Ala Gln Ser Ile Arg Gln Ser Gly Ser His Lys Asp Met Ile | |
| 290 295 300 | |
| ctc ctc cat gat cat acc ata acc aac aag tct ctt att ggt ctc agc | 960 |
| Leu Leu His Asp His Thr Ile Thr Asn Lys Ser Leu Ile Gly Leu Ser | |
| 305 310 315 320 | |
| gct gcg gga tgg aat ctc cgg cta atc gac agg atc cgc agt cct ttt | 1008 |
| Ala Ala Gly Trp Asn Leu Arg Leu Ile Asp Arg Ile Arg Ser Pro Phe | |
| 325 330 335 | |
| tcg caa aaa gac tct tat aat gag tgg aac tat agc aaa tta cgt gtg | 1056 |
| Ser Gln Lys Asp Ser Tyr Asn Glu Trp Asn Tyr Ser Lys Leu Arg Val | |
| 340 345 350 | |
| tgg caa gta act gac tac gat aaa ctt gtg ttc ata gac gca gat ttc | 1104 |
| Trp Gln Val Thr Asp Tyr Asp Lys Leu Val Phe Ile Asp Ala Asp Phe | |
| 355 360 365 | |
| atc atc ctc aag aaa ctt gat cat ctc ttc tac tat cca caa ctc tca | 1152 |
| Ile Ile Leu Lys Lys Leu Asp His Leu Phe Tyr Tyr Pro Gln Leu Ser | |
| 370 375 380 | |
| gct tca ggc aac gac aaa gtg tta ttc aac tcc gga atc atg gtt ctc | 1200 |
| Ala Ser Gly Asn Asp Lys Val Leu Phe Asn Ser Gly Ile Met Val Leu | |
| 385 390 395 400 | |
| gag cca tcg gca tgt atg ttt aaa gat tta atg gag aaa tcg ttc aag | 1248 |
| Glu Pro Ser Ala Cys Met Phe Lys Asp Leu Met Glu Lys Ser Phe Lys | |
| 405 410 415 | |

| | | |
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| att gag tca tac aac gga gga gac caa gga ttc ctt aat gag ata ttt | | 1296 |
| Ile Glu Ser Tyr Asn Gly Gly Asp Gln Gly Phe Leu Asn Glu Ile Phe | | |
| 420 | 425 | 430 |
| gta tgg tgg cac agg tta tcg aaa cga gtg aac aca atg aag tac ttc | | 1344 |
| Val Trp Trp His Arg Leu Ser Lys Arg Val Asn Thr Met Lys Tyr Phe | | |
| 435 | 440 | 445 |
| gac gaa aaa aat cat cga aga cac gat ctt cct gag aat gta gaa ggt | | 1392 |
| Asp Glu Lys Asn His Arg Arg His Asp Leu Pro Glu Asn Val Glu Gly | | |
| 450 | 455 | 460 |
| ctg cac tac ttg ggg ttg aaa cca tgg gta tgt tat aga gac tat gat | | 1440 |
| Leu His Tyr Leu Gly Leu Lys Pro Trp Val Cys Tyr Arg Asp Tyr Asp | | |
| 465 | 470 | 475 |
| 480 | | |
| tgc aat tgg gac att agc gaa cga cgc gtg ttt gca agc gat tct gtg | | 1488 |
| Cys Asn Trp Asp Ile Ser Glu Arg Arg Val Phe Ala Ser Asp Ser Val | | |
| 485 | 490 | 495 |
| cac gaa aaa tgg tgg aaa gtg tat gac aaa atg tca gag cag ttg aaa | | 1536 |
| His Glu Lys Trp Trp Lys Val Tyr Asp Lys Met Ser Glu Gln Leu Lys | | |
| 500 | 505 | 510 |
| ggt tat tgt ggt ttg aat aag aat atg gag aag agg att gag aag tgg | | 1584 |
| Gly Tyr Cys Gly Leu Asn Lys Asn Met Glu Lys Arg Ile Glu Lys Trp | | |
| 515 | 520 | 525 |
| aga aga atc gct aag aac aat agt ttg cct gat agg cat tgg gag att | | 1632 |
| Arg Arg Ile Ala Lys Asn Asn Ser Leu Pro Asp Arg His Trp Glu Ile | | |
| 530 | 535 | 540 |
| gaa gtg aga gat cct agg aag acg aat ctt ctt gtt cag tga | | 1674 |
| Glu Val Arg Asp Pro Arg Lys Thr Asn Leu Leu Val Gln | | |
| 545 | 550 | 555 |
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| <211> 557 | | |
| <212> PRT | | |
| <213> <i>Arabidopsis thaliana</i> | | |
| <400> 11 | | |
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| Leu Ile Leu Val Ser Leu Ser Leu Leu Gly Leu Ile Leu Pro Phe Lys | | |
| 20 | 25 | 30 |
| Pro Leu Phe Arg Ile Thr Ser Pro Ser Ser Thr Leu Arg Ile Asp Leu | | |
| 35 | 40 | 45 |
| Pro Ser Pro Gln Val Asn Lys Asn Pro Lys Trp Leu Arg Leu Ile Arg | | |
| 50 | 55 | 60 |

26

Asn Tyr Leu Pro Glu Lys Arg Ile Gln Val Gly Phe Leu Asn Ile Asp
 65 70 75 80
 Glu Lys Glu Arg Glu Ser Tyr Glu Ala Arg Gly Pro Leu Val Leu Lys
 85 90 95
 Asn Ile His Val Pro Leu Asp His Ile Pro Lys Asn Val Thr Trp Lys
 100 105 110
 Ser Leu Tyr Pro Glu Trp Ile Asn Glu Glu Ala Ser Thr Cys Pro Glu
 115 120 125
 Ile Pro Leu Pro Gln Pro Glu Gly Ser Asp Ala Asn Val Asp Val Ile
 130 135 140
 Val Ala Arg Val Pro Cys Asp Gly Trp Ser Ala Asn Lys Gly Leu Arg
 145 150 155 160
 Asp Val Phe Arg Leu Gln Val Asn Leu Ala Ala Asn Leu Ala Val
 165 170 175
 Gln Ser Gly Leu Arg Thr Val Asn Gln Ala Val Tyr Val Val Phe Ile
 180 185 190
 Gly Ser Cys Gly Pro Met His Glu Ile Phe Pro Cys Asp Glu Arg Val
 195 200 205
 Met Arg Val Glu Asp Tyr Trp Val Tyr Lys Pro Tyr Leu Pro Arg Leu
 210 215 220
 Lys Gln Lys Leu Leu Met Pro Val Gly Ser Cys Gln Ile Ala Pro Ser
 225 230 235 240
 Phe Ala Gln Phe Gly Gln Glu Ala Trp Arg Pro Lys His Glu Asp Asn
 245 250 255
 Leu Ala Ser Lys Ala Val Thr Ala Leu Pro Arg Arg Leu Arg Val Ala
 260 265 270
 Tyr Val Thr Val Leu His Ser Ser Glu Ala Tyr Val Cys Gly Ala Ile
 275 280 285
 Ala Leu Ala Gln Ser Ile Arg Gln Ser Gly Ser His Lys Asp Met Ile
 290 295 300
 Leu Leu His Asp His Thr Ile Thr Asn Lys Ser Leu Ile Gly Leu Ser
 305 310 315 320
 Ala Ala Gly Trp Asn Leu Arg Leu Ile Asp Arg Ile Arg Ser Pro Phe
 325 330 335
 Ser Gln Lys Asp Ser Tyr Asn Glu Trp Asn Tyr Ser Lys Leu Arg Val
 340 345 350
 Trp Gln Val Thr Asp Tyr Asp Lys Leu Val Phe Ile Asp Ala Asp Phe
 355 360 365
 Ile Ile Leu Lys Lys Leu Asp His Leu Phe Tyr Tyr Pro Gln Leu Ser
 370 375 380
 Ala Ser Gly Asn Asp Lys Val Leu Phe Asn Ser Gly Ile Met Val Leu
 385 390 395 400
 Glu Pro Ser Ala Cys Met Phe Lys Asp Leu Met Glu Lys Ser Phe Lys
 405 410 415
 Ile Glu Ser Tyr Asn Gly Gly Asp Gln Gly Phe Leu Asn Glu Ile Phe
 420 425 430
 Val Trp Trp His Arg Leu Ser Lys Arg Val Asn Thr Met Lys Tyr Phe
 435 440 445
 Asp Glu Lys Asn His Arg Arg His Asp Leu Pro Glu Asn Val Glu Gly
 450 455 460
 Leu His Tyr Leu Gly Leu Lys Pro Trp Val Cys Tyr Arg Asp Tyr Asp
 465 470 475 480

27

Cys Asn Trp Asp Ile Ser Glu Arg Arg Val Phe Ala Ser Asp Ser Val
 485 490 495
 His Glu Lys Trp Trp Lys Val Tyr Asp Lys Met Ser Glu Gln Leu Lys
 500 505 510
 Gly Tyr Cys Gly Leu Asn Lys Asn Met Glu Lys Arg Ile Glu Lys Trp
 515 520 525
 Arg Arg Ile Ala Lys Asn Asn Ser Leu Pro Asp Arg His Trp Glu Ile
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 Glu Val Arg Asp Pro Arg Lys Thr Asn Leu Leu Val Gln
 545 550 555

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 1 5 10 15
 gca ggt gta gac aaa gtt tat gac cta acg aag ata gaa gca gag aca 96
 Ala Gly Val Asp Lys Val Tyr Asp Leu Thr Lys Ile Glu Ala Glu Thr
 20 25 30
 aaa cga cca aaa cgt gaa gcc tac gta act gtt ctt cac tct tcc gag 144
 Lys Arg Pro Lys Arg Glu Ala Tyr Val Thr Val Leu His Ser Ser Glu
 35 40 45
 tct tat gtc tgt ggt gcc ata act ttg gct caa agc ctc ctt cag aca 192
 Ser Tyr Val Cys Gly Ala Ile Thr Leu Ala Gln Ser Leu Leu Gln Thr
 50 55 60
 aac acc aaa cgc gat ctt atc ctt ctc cac gat gac tcc atc tcc att 240
 Asn Thr Lys Arg Asp Leu Ile Leu His Asp Asp Ser Ile Ser Ile
 65 70 75 80
 acc aaa ctt cga gct ctc gcc gca gga tgg aag ctt cgt cgg atc 288
 Thr Lys Leu Arg Ala Leu Ala Ala Gly Trp Lys Leu Arg Arg Ile
 85 90 95
 att cga atc aga aac cca ctt gcg gag aag gac tcg tac aat gaa tac 336
 Ile Arg Ile Arg Asn Pro Leu Ala Glu Lys Asp Ser Tyr Asn Glu Tyr
 100 105 110
 aac tac agc aag ttt cga ctc tgg caa ttg aca gat tac gac aaa gtg 384

28

| | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|-----|
| Asn | Tyr | Ser | Lys | Phe | Arg | Leu | Trp | Gln | Leu | Thr | Asp | Tyr | Asp | Lys | Val | |
| 115 | | | | | | 120 | | | | | 125 | | | | | |
| atc | tcc | att | gat | gcc | gac | atc | atc | gtc | tta | cgt | aac | ctt | gat | ctt | ctc | 432 |
| Ile | Phe | Ile | Asp | Ala | Asp | Ile | Ile | Val | Ile | Arg | Asn | Leu | Asp | Leu | Leu | |
| 130 | | | | | | 135 | | | | | 140 | | | | | |
| ttc | cat | ttt | cct | cag | atg | tcg | gcc | acc | gga | aat | gat | gta | tgg | ata | tat | 480 |
| Phe | His | Phe | Pro | Gln | Met | Ser | Ala | Thr | Gly | Asn | Asp | Val | Trp | Ile | Tyr | |
| 145 | | | | | 150 | | | | 155 | | | 160 | | | | |
| aat | tca | ggc | atc | atg | gtc | atc | gag | cct | tct | aat | tgt | acg | ttt | act | aca | 528 |
| Asn | Ser | Gly | Ile | Met | Val | Ile | Glu | Pro | Ser | Asn | Cys | Thr | Phe | Thr | Thr | |
| 165 | | | | | 170 | | | | 175 | | | | | | | |
| atc | atg | agc | cag | cga | agc | gag | atc | gtt | tca | tac | aac | ggt | gga | gat | caa | 576 |
| Ile | Met | Ser | Gln | Arg | Ser | Glu | Ile | Val | Ser | Tyr | Asn | Gly | Gly | Asp | Gln | |
| 180 | | | | | 185 | | | 190 | | | | | | | | |
| ggg | tac | cta | aac | gag | ata | ttt | gtg | tgg | tgg | cac | cga | ttg | cct | cga | cga | 624 |
| Gly | Tyr | Leu | Asn | Glu | Ile | Phe | Val | Trp | Trp | His | Arg | Leu | Pro | Arg | Arg | |
| 195 | | | | | 200 | | | 205 | | | | | | | | |
| gta | aac | ttt | ctg | aag | aac | tcc | tgg | tcg | aac | aca | acc | aaa | gaa | aga | aac | 672 |
| Val | Asn | Phe | Leu | Lys | Asn | Phe | Trp | Ser | Asn | Thr | Thr | Lys | Glu | Arg | Asn | |
| 210 | | | | | 215 | | | 220 | | | | | | | | |
| atc | aag | aac | aac | ctc | tcc | gcc | gcg | gag | ccg | cct | cag | gtc | tac | gcf | gtc | 720 |
| Ile | Lys | Asn | Asn | Leu | Phe | Ala | Ala | Glu | Pro | Pro | Gln | Val | Tyr | Ala | Val | |
| 225 | | | | 230 | | | | 235 | | | 240 | | | | | |
| cac | tac | tta | ggt | tgg | aaa | cca | tgg | ctt | tgc | tat | agg | gac | tac | gat | tgc | 768 |
| His | Tyr | Leu | Gly | Trp | Lys | Pro | Trp | Leu | Cys | Tyr | Arg | Asp | Tyr | Asp | Cys | |
| 245 | | | | | 250 | | | 255 | | | | | | | | |
| aac | tac | gac | gtg | gac | gag | cag | ttg | gtg | tac | gct | agt | gat | gcf | gct | cac | 816 |
| Asn | Tyr | Asp | Val | Asp | Glu | Gln | Leu | Val | Tyr | Ala | Ser | Asp | Ala | Ala | His | |
| 260 | | | | | 265 | | | 270 | | | | | | | | |
| gtt | agg | tgg | tgg | aaa | gtg | cac | gac | tcc | atg | gac | gat | gca | ttg | caa | aag | 864 |
| Val | Arg | Trp | Trp | Lys | Val | His | Asp | Ser | Met | Asp | Asp | Ala | Leu | Gln | Lys | |
| 275 | | | | | 280 | | | 285 | | | | | | | | |
| ttt | tgc | agg | ctg | acg | aaa | aag | agg | aga | acg | gag | atc | aac | tgg | gag | agg | 912 |
| Phe | Cys | Arg | Leu | Thr | Lys | Lys | Arg | Arg | Thr | Glu | Ile | Asn | Trp | Glu | Arg | |
| 290 | | | | | 295 | | | 300 | | | | | | | | |
| agg | aaa | gca | agg | ctt | aga | ggt | tcc | act | gat | tat | cat | tgg | aag | atc | aat | 960 |
| Arg | Lys | Ala | Arg | Leu | Arg | Gly | Ser | Thr | Asp | Tyr | His | Trp | Lys | Ile | Asn | |
| 305 | | | | | 310 | | | 315 | | | 320 | | | | | |
| gtc | act | gat | cca | aga | cga | cgt | cgt | tct | tat | ttg | att | ggt | taa | | 1002 | |

29

Val Thr Asp Pro Arg Arg Arg Ser Tyr Leu Ile Gly
 325 330

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 <211> 333
 <212> PRT
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 Ala Gly Val Asp Lys Val Tyr Asp Leu Thr Lys Ile Glu Ala Glu Thr
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 Lys Arg Pro Lys Arg Glu Ala Tyr Val Thr Val Leu His Ser Ser Glu
 35 40 45
 Ser Tyr Val Cys Gly Ala Ile Thr Leu Ala Gln Ser Leu Leu Gln Thr
 50 55 60
 Asn Thr Lys Arg Asp Leu Ile Leu His Asp Asp Ser Ile Ser Ile
 65 70 75 80
 Thr Lys Leu Arg Ala Leu Ala Ala Gly Trp Lys Leu Arg Arg Ile
 85 90 95
 Ile Arg Ile Arg Asn Pro Leu Ala Glu Lys Asp Ser Tyr Asn Glu Tyr
 100 105 110
 Asn Tyr Ser Lys Phe Arg Leu Trp Gln Leu Thr Asp Tyr Asp Lys Val
 115 120 125
 Ile Phe Ile Asp Ala Asp Ile Ile Val Leu Arg Asn Leu Asp Leu Leu
 130 135 140
 Phe His Phe Pro Gln Met Ser Ala Thr Gly Asn Asp Val Trp Ile Tyr
 145 150 155 160
 Asn Ser Gly Ile Met Val Ile Glu Pro Ser Asn Cys Thr Phe Thr Thr
 165 170 175
 Ile Met Ser Gln Arg Ser Glu Ile Val Ser Tyr Asn Gly Gly Asp Gln
 180 185 190
 Gly Tyr Leu Asn Glu Ile Phe Val Trp Trp His Arg Leu Pro Arg Arg
 195 200 205
 Val Asn Phe Leu Lys Asn Phe Trp Ser Asn Thr Thr Lys Glu Arg Asn
 210 215 220
 Ile Lys Asn Asn Leu Phe Ala Ala Glu Pro Pro Gln Val Tyr Ala Val
 225 230 235 240
 His Tyr Leu Gly Trp Lys Pro Trp Leu Cys Tyr Arg Asp Tyr Asp Cys
 245 250 255
 Asn Tyr Asp Val Asp Glu Gln Leu Val Tyr Ala Ser Asp Ala Ala His
 260 265 270
 Val Arg Trp Trp Lys Val His Asp Ser Met Asp Asp Ala Leu Gln Lys
 275 280 285
 Phe Cys Arg Leu Thr Lys Lys Arg Arg Thr Glu Ile Asn Trp Glu Arg
 290 295 300
 Arg Lys Ala Arg Leu Arg Gly Ser Thr Asp Tyr His Trp Lys Ile Asn
 305 310 315 320
 Val Thr Asp Pro Arg Arg Arg Ser Tyr Leu Ile Gly
 325 330

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 <211> 834
 <212> DNA
 <213> *Arabidopsis thaliana*

<220>
 <221> CDS
 <222> (1)...(834)

<400> 14

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| atg | gct | cct | tcc | aaa | tct | gca | ctg | ata | cgc | ttt | aat | cta | gtc | ttg | ttg | 48 |
| Met | Ala | Pro | Ser | Lys | Ser | Ala | Leu | Ile | Arg | Phe | Asn | Leu | Val | Leu | Leu | |
| 1 | 5 | | | | | 10 | | | | | 15 | | | | | |

gca gcg gag ctt cct ttg ttg gat gct ctt ttc gtg att gca ctc cca 96

| | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
| Ala | Ala | Glu | Leu | Pro | Leu | Leu | Asp | Ala | Leu | Phe | Val | Ile | Ala | Leu | Pro | |
| 20 | 25 | | | | | | | 30 | | | | | | | | |

aga cta ata gat atc ttt ata ctg cta tgt gat cag gtg gtg aga gga 144

| | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
| Arg | Leu | Ile | Asp | Ile | Phe | Ile | Leu | Leu | Cys | Asp | Gln | Val | Val | Arg | Gly | |
| 35 | 40 | | | | | | | 45 | | | | | | | | |

gtg aag atg caa gaa ctc gtt gaa gag aac gaa ata aac aag aaa gat 192

| | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
| Val | Lys | Met | Gln | Glu | Leu | Val | Glu | Glu | Asn | Glu | Ile | Asn | Lys | Lys | Asp | |
| 50 | 55 | | | | | 60 | | | | | | | | | | |

ttg cta acc gct agt aac cag aca aag ctg gag gcg cca agc ttc atg 240

| | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
| Leu | Leu | Thr | Ala | Ser | Asn | Gln | Thr | Lys | Leu | Glu | Ala | Pro | Ser | Phe | Met | |
| 65 | 70 | | | | | | 75 | | | | 80 | | | | | |

gaa gag att tta aca aga ggg tta gga aaa aca aag ata ggg atg gtg 288

| | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
| Glu | Glu | Ile | Leu | Thr | Arg | Gly | Leu | Gly | Lys | Thr | Lys | Ile | Gly | Met | Val | |
| 85 | 90 | | | | | | 95 | | | | | | | | | |

aac atg gaa gaa tgt gat ctt act aat tgg aaa cgt tat ggc gaa acg 336

| | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
| Asn | Met | Glu | Glu | Cys | Asp | Leu | Thr | Asn | Trp | Lys | Arg | Tyr | Gly | Glu | Thr | |
| 100 | 105 | | | | | | 110 | | | | | | | | | |

gtt cac ata cat ttt gag cgt gtc tcg aag ctc ttc aaa tgg caa gac 384

| | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
| Val | His | Ile | His | Phe | Glu | Arg | Val | Ser | Lys | Leu | Phe | Lys | Trp | Gln | Asp | |
| 115 | 120 | | | | | | 125 | | | | | | | | | |

ttg ttc ccc gag tgg ata gat gaa gag gaa acc gag gtt ccc aca 432

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
| Leu | Phe | Pro | Glu | Trp | Ile | Asp | Glu | Glu | Glu | Thr | Glu | Val | Pro | Thr | |
| 130 | 135 | | | | | | 140 | | | | | | | | |

tgt cct gag ata cct atg ccc gat ttc gaa agc tta gag aag ttg gat 480

| | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
| Cys | Pro | Glu | Ile | Pro | Met | Pro | Asp | Phe | Glu | Ser | Leu | Glu | Lys | Leu | Asp | |
| 145 | 150 | | | | | | 155 | | | | 160 | | | | | |

ttg gta gta gtg aag ttg cct tgt aat tac cct gaa gaa ggg tgg aga 528
 Leu Val Val Val Lys Leu Pro Cys Asn Tyr Pro Glu Glu Gly Trp Arg
 165 170 175

aga gag gtt ttg agg ttg caa gtg aac cta gtt gcg gct aac ttg gca 576
 Arg Glu Val Leu Arg Leu Gln Val Asn Leu Val Ala Ala Asn Leu Ala
 180 185 190

gcc aag aaa ggg aag acg gat tgg aga tgg aaa agc aaa gtg ttg ttt 624
 Ala Lys Lys Gly Lys Thr Asp Trp Arg Trp Lys Ser Lys Val Leu Phe
 195 200 205

tgg agc aaa tgt caa ccg atg att gag att ttc cgg tgt gat gat ttg 672
 Trp Ser Lys Cys Gln Pro Met Ile Glu Ile Phe Arg Cys Asp Asp Leu
 210 215 220

gag aag aga gag gca gat tgg tgg ctg tat cgc cct gag gtg gtt agg 720
 Glu Lys Arg Glu Ala Asp Trp Trp Leu Tyr Arg Pro Glu Val Val Arg
 225 230 235 240

tta caa cag aga ctc agt ttg cca gtc gga tct tgc aat ctt gct ctt 768
 Leu Gln Gln Arg Leu Ser Leu Pro Val Gly Ser Cys Asn Leu Ala Leu
 245 250 255

cct ttg tgg gca cca caa ggt aaa att act ttc atg caa att aat ctt 816
 Pro Leu Trp Ala Pro Gln Gly Lys Ile Thr Phe Met Gln Ile Asn Leu
 260 265 270

ctt gct aaa tat ttt tag 834
 Leu Ala Lys Tyr Phe
 275

<210> 15
 <211> 277
 <212> PRT
 <213> *Arabidopsis thaliana*

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 Met Ala Pro Ser Lys Ser Ala Leu Ile Arg Phe Asn Leu Val Leu Leu
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 Ala Ala Glu Leu Pro Leu Leu Asp Ala Leu Phe Val Ile Ala Leu Pro
 20 25 30

Arg Leu Ile Asp Ile Phe Ile Leu Leu Cys Asp Gln Val Val Arg Gly
 35 40 45

Val Lys Met Gln Glu Leu Val Glu Glu Asn Glu Ile Asn Lys Lys Asp
 50 55 60

Leu Leu Thr Ala Ser Asn Gln Thr Lys Leu Glu Ala Pro Ser Phe Met
 65 70 75 80

Glu Glu Ile Leu Thr Arg Gly Leu Gly Lys Thr Lys Ile Gly Met Val
 85 90 95

32

Asn Met Glu Glu Cys Asp Leu Thr Asn Trp Lys Arg Tyr Gly Glu Thr
 100 105 110
 Val His Ile His Phe Glu Arg Val Ser Lys Leu Phe Lys Trp Gln Asp
 115 120 125
 Leu Phe Pro Glu Trp Ile Asp Glu Glu Glu Thr Glu Val Pro Thr
 130 135 140
 Cys Pro Glu Ile Pro Met Pro Asp Phe Glu Ser Leu Glu Lys Leu Asp
 145 150 155 160
 Leu Val Val Val Lys Leu Pro Cys Asn Tyr Pro Glu Glu Gly Trp Arg
 165 170 175
 Arg Glu Val Leu Arg Leu Gln Val Asn Leu Val Ala Ala Asn Leu Ala
 180 185 190
 Ala Lys Lys Gly Lys Thr Asp Trp Arg Trp Lys Ser Lys Val Leu Phe
 195 200 205
 Trp Ser Lys Cys Gln Pro Met Ile Glu Ile Phe Arg Cys Asp Asp Leu
 210 215 220
 Glu Lys Arg Glu Ala Asp Trp Trp Leu Tyr Arg Pro Glu Val Val Arg
 225 230 235 240
 Leu Gln Gln Arg Leu Ser Leu Pro Val Gly Ser Cys Asn Leu Ala Leu
 245 250 255
 Pro Leu Trp Ala Pro Gln Gly Lys Ile Thr Phe Met Gln Ile Asn Leu
 260 265 270
 Leu Ala Lys Tyr Phe
 275

<210> 16
 <211> 383
 <212> DNA
 <213> Hordeum vulgare

<220>
 <221> CDS
 <222> (46)..(381)

<400> 16
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 Pro Glu Ala Glu
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cgt gat gct tac aat gag tgg aac tac agc aag ttc cgg ttg tgg cag 105
 Arg Asp Ala Tyr Asn Glu Trp Asn Tyr Ser Lys Phe Arg Leu Trp Gln
 5 10 15 20

ctc acg gac tat gac aag atc ata ttc ata gat gct gat ctg ctc atc 153
 Leu Thr Asp Tyr Asp Lys Ile Ile Phe Ile Asp Ala Asp Leu Leu Ile
 25 30 35

ttg agg aac att gat ttc ctg ttt aca atg cca gaa atc agt gca acc 201
 Leu Arg Asn Ile Asp Phe Leu Phe Thr Met Pro Glu Ile Ser Ala Thr
 40 45 50

ggc aac aat gca aca ctc ttc aac tct ggt gtc atg gtc atc gaa ccc 249
 Gly Asn Asn Ala Thr Leu Phe Asn Ser Gly Val Met Val Ile Glu Pro
 55 60 65

tca aac tgc aca ttc cag ctg tta atg gag cac atc aat gag ata aca 297
 Ser Asn Cys Thr Phe Gln Leu Leu Met Glu His Ile Asn Glu Ile Thr
 70 75 80

tct tac aat ggt ggt gat cag ggc tac ttg aat gag ata ttc aca tgg 345
 Ser Tyr Asn Gly Gly Asp Gln Gly Tyr Leu Asn Glu Ile Phe Thr Trp
 85 90 95 100

tgg cat cgg att ccc aag cac atg aac ttc ctg aag ca 383
 Trp His Arg Ile Pro Lys His Met Asn Phe Leu Lys
 105 110

<210> 17
 <211> 112
 <212> PRT
 <213> Hordeum vulgare

<400> 17
 Pro Glu Ala Glu Arg Asp Ala Tyr Asn Glu Trp Asn Tyr Ser Lys Phe
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Arg Leu Trp Gln Leu Thr Asp Tyr Asp Lys Ile Ile Phe Ile Asp Ala
 20 25 30

Asp Leu Leu Ile Leu Arg Asn Ile Asp Phe Leu Phe Thr Met Pro Glu
 35 40 45

Ile Ser Ala Thr Gly Asn Asn Ala Thr Leu Phe Asn Ser Gly Val Met
 50 55 60

Val Ile Glu Pro Ser Asn Cys Thr Phe Gln Leu Leu Met Glu His Ile
 65 70 75 80

Asn Glu Ile Thr Ser Tyr Asn Gly Gly Asp Gln Gly Tyr Leu Asn Glu
 85 90 95

Ile Phe Thr Trp Trp His Arg Ile Pro Lys His Met Asn Phe Leu Lys
 100 105 110

<210> 18
 <211> 245
 <212> DNA
 <213> Hordeum vulgare

<220>

<221> CDS

<222> (52)..(243)

<400> 18

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 Pro Glu
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gcc gag cgt gat gct tac aat gag tgg aac tac agc aag ttc cgg ttg 105
 Ala Glu Arg Asp Ala Tyr Asn Glu Trp Asn Tyr Ser Lys Phe Arg Leu
 5 10 15

tgg cag ctc acg gac tat gac aag atc ata ttc ata gat gct gat ctg 153
 Trp Gln Leu Thr Asp Tyr Asp Lys Ile Ile Phe Ile Asp Ala Asp Leu
 20 25 30

ctc atc ttg agg aac att gat ttc ctg ttt aca atg cca gaa atc agt 201
 Leu Ile Leu Arg Asn Ile Asp Phe Leu Phe Thr Met Pro Glu Ile Ser
 35 40 45 50

gca aac ggc aac aat gca aca ctc ttc aac tct ggt gtc atg gt 245
 Ala Asn Gly Asn Asn Ala Thr Leu Phe Asn Ser Gly Val Met
 55 60

<210> 19

<211> 64

<212> PRT

<213> Hordeum vulgare

<400> 19

Pro Glu Ala Glu Arg Asp Ala Tyr Asn Glu Trp Asn Tyr Ser Lys Phe
 1 5 10 15

Arg Leu Trp Gln Leu Thr Asp Tyr Asp Lys Ile Ile Phe Ile Asp Ala
 20 25 30

Asp Leu Leu Ile Leu Arg Asn Ile Asp Phe Leu Phe Thr Met Pro Glu
 35 40 45

Ile Ser Ala Asn Gly Asn Asn Ala Thr Leu Phe Asn Ser Gly Val Met
 50 55 60

<210> 20

<211> 1284

<212> DNA

<213> Triticum aestivum

<220>

<221> CDS

<222> (1)..(1284)

<400> 20

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Thr Arg Pro Leu Ala Phe Phe Phe Leu Val Leu His Gly Pro Pro Ala
1 5 10 15

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cca ccc caa gta ctc cca cat cct cga ccg cgg cgc ctc ctc tct ggt 96
Pro Pro Gln Val Leu Pro His Pro Arg Pro Arg Arg Leu Leu Ser Gly
20          25          30

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ccg ctg cac ctt ccg cga cgc ctg ccc gtc cac gtc cca cct ctc acg 144
 Pro Leu His Leu Pro Arg Arg Leu Pro Val His Val Pro Pro Leu Thr
 35 40 45

| | |
|---|-----|
| gaa ggt aag ccg gga gga aga tca gtg gcg gcg gcg aac aag gtg gtg | 192 |
| Glu Gly Lys Pro Gly Gly Arg Ser Val Ala Ala Ala Asn Lys Val Val | |
| 50 55 60 | |

gcg acg gag cggtt atc gtg aac gcg ggg cgc gcg ccg acc atg ttc aac 240
 Ala Thr Glu Arg Ile Val Asn Ala Gly Arg Ala Pro Thr Met Phe Asn
 65 70 75 80

gag ctg cgc ggc cgg ctg cgg atg ggc ctg gtg aac atc ggc cgc gac 288
 Glu Leu Arg Gly Arg Leu Arg Met Gly Leu Val Asn Ile Gly Arg Asp
 85 90 95

gag ctg ctg gcg ctg ggc gtg gag gga gac gcc gtg ggc gtg gac ttc 336
 Glu Leu Leu Ala Leu Gly Val Glu Gly Asp Ala Val Gly Val Asp Phe
 . 100 105 110

gac cgc gtg tcg gac gtg ttc cg^g tgg tca gac ctg ttc ccg gag tgg 384
 Asp Arg Val Ser Asp Val Phe Arg Trp Ser Asp Leu Phe Pro Glu Trp
 115 120 125

atc gac gag gag gag gag gac ggc gtc ccc tcc tgc ccc gag atc ccc 432
 Ile Asp Glu Glu Glu Glu Asp Gly Val Pro Ser Cys Pro Glu Ile Pro
 130 135 140

```

atg ccg gac ttc tcc cggtac gac gac gac ggc gtg gac gtg gtg gtg 480
Met Pro Asp Phe Ser Arg Tyr Asp Asp Asp Gly Val Asp Val Val Val
145           150           155           160

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gcg gcg ctg ccg tgc aac cgg acg gcg gtc cgg ggg tgg aac cgc gac 528
Ala Ala Leu Pro Cys Asn Arg Thr Ala Val Arg Gly Trp Asn Arg Asp
165 170 175

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gtg ttc agg. ctg cag gtg cac ctg gtg gcg gcg cac atg gcg gcg cg 576
 Val Phe Arg Leu Gln Val His Leu Val Ala Ala His Met Ala Ala Arg
 180 185 190

36

| | | | |
|---------------------------------|---------------------|---------------------|------|
| aag tgg gcg gcg cga cgg cgc cgg | ggg ggt gcg cgt | ggt gct gcg gag | 624 |
| Lys Trp Ala Ala Arg Arg Arg Arg | Pro Gly Ala Arg Gly | Ala Ala Glu | |
| 195 | 200 | 205 | |
| cga gtg cga gcc gat gat | gga cct gtt ccg gtg | cga cga gtc cgt | 672 |
| Arg Val Arg Ala Asp Asp Gly | Pro Val Pro Val | Arg Arg Val Arg Gly | |
| 210 | 215 | 220 | |
| gcg gga ggg gga ctg gtg gat | gta cag cgt cga cgc | gcc gcg cat gga | 720 |
| Ala Gly Gly Leu Val Asp Val | Gln Arg Arg Arg | Ala Ala His Gly | |
| 225 | 230 | 235 | 240 |
| gga gaa gct ccg gct gcc cat | cgg ctc ctg caa cct | ccg cgc tgc cgc | 768 |
| Gly Glu Ala Pro Ala Ala His | Arg Leu Leu Gln | Pro Arg Arg Cys Arg | |
| 245 | 250 | 255 | |
| tct ggg ggc caa cag gca tcc acg | agg tgt tca acg cgt | cag acc taa | 816 |
| Ser Gly Gly Gln Ala Ser Thr | Arg Cys Ser Thr Arg | Gln Thr | |
| 260 | 265 | 270 | |
| cag cgg tgg acg ccg gca gcc | agc ggc agg cgt acg | cga ctg gtg | 864 |
| Gln Arg Trp Thr Pro Ala Ala Ser | Gly Ala Arg Arg | Thr Arg Leu Val | |
| 275 | 280 | 285 | |
| ctg cac tcg tcc gac cga tac | ctg tgc ggc gcc atc | gtg ctg gcg cag | 912 |
| Leu His Ser Ser Asp Arg Tyr | Leu Cys Gly Ala Ile | Val Leu Ala Gln | |
| 290 | 295 | 300 | |
| agc atc cgg cgg tcg ggc tcc | acc cgc gac atg gtc | ctc ctc cac gac | 960 |
| Ser Ile Arg Arg Ser Gly Ser | Thr Arg Asp Met Val | Leu Leu His Asp | |
| 305 | 310 | 315 | 320 |
| cac acc gtc tcc aag ccg gcc | ctc cgc gcg ctg gtc | gcc gcc ggc tgg | 1008 |
| His Thr Val Ser Lys Pro Ala | Leu Arg Ala Leu Val | Ala Ala Gly Trp | |
| 325 | 330 | 335 | |
| atc ccg cgc agg atc ccg cgc | atc cgc aac ccg cgc | gag cgg ggc | 1056 |
| Ile Pro Arg Arg Ile Arg Arg | Ile Arg Asn Pro Arg | Ala Glu Arg Gly | |
| 340 | 345 | 350 | |
| tcc tac aac gag tac aac tac | agc aag ttc ccg | ctg tgg cag ctg acg | 1104 |
| Ser Tyr Asn Glu Tyr Asn Tyr | Ser Lys Phe Arg | Leu Trp Gln Leu Thr | |
| 355 | 360 | 365 | |
| gag tac ttc cgc gtc gtc | ttc atc gac gcc | gac atc ctc gtc | 1152 |
| Glu Tyr Phe Arg Val Val | Phe Ile Asp Ala Asp | Ile Leu Val Leu Arg | |
| 370 | 375 | 380 | |
| tcc ctc gac gcg ctc ttc cgc | ttc ccg cag atc | tcc gcc ggg ggc aac | 1200 |
| Ser Leu Asp Ala Leu Phe Arg | Phe Pro Gln Ile | Ser Ala Gly Gly Asn | |
| 385 | 390 | 395 | 400 |

37

gac ggc tcc ctc ttc aac tcg ggg aac atg gtg ctc gag ccg tcg gcg 1248
 Asp Gly Ser Leu Phe Asn Ser Gly Asn Met Val Leu Glu Pro Ser Ala
 405 410 415

tgc acc ttc gag gcg ctc gtc cg^g ggg cg^g cg^c aca 1284
 Cys Thr Phe Glu Ala Leu Val Arg Gly Arg Arg Thr
 420 425

<210> 21
 <211> 271
 <212> PRT
 <213> *Triticum aestivum*

<400> 21
 Thr Arg Pro Leu Ala Phe Phe Leu Val Leu His Gly Pro Pro Ala
 1 5 10 15
 Pro Pro Gln Val Leu Pro His Pro Arg Pro Arg Arg Leu Leu Ser Gly
 20 25 30
 Pro Leu His Leu Pro Arg Arg Leu Pro Val His Val Pro Pro Leu Thr
 35 40 45
 Glu Gly Lys Pro Gly Gly Arg Ser Val Ala Ala Ala Asn Lys Val Val
 50 55 60
 Ala Thr Glu Arg Ile Val Asn Ala Gly Arg Ala Pro Thr Met Phe Asn
 65 70 75 80
 Glu Leu Arg Gly Arg Leu Arg Met Gly Leu Val Asn Ile Gly Arg Asp
 85 90 95
 Glu Leu Leu Ala Leu Gly Val Glu Gly Asp Ala Val Gly Val Asp Phe
 100 105 110
 Asp Arg Val Ser Asp Val Phe Arg Trp Ser Asp Leu Phe Pro Glu Trp
 115 120 125
 Ile Asp Glu Glu Glu Asp Gly Val Pro Ser Cys Pro Glu Ile Pro
 130 135 140
 Met Pro Asp Phe Ser Arg Tyr Asp Asp Asp Gly Val Asp Val Val Val
 145 150 155 160
 Ala Ala Leu Pro Cys Asn Arg Thr Ala Val Arg Gly Trp Asn Arg Asp
 165 170 175
 Val Phe Arg Leu Gln Val His Leu Val Ala Ala His Met Ala Ala Arg
 180 185 190
 Lys Trp Ala Ala Arg Arg Arg Pro Gly Ala Arg Gly Ala Ala Glu
 195 200 205
 Arg Val Arg Ala Asp Asp Gly Pro Val Pro Val Arg Arg Val Arg Gly
 210 215 220
 Ala Gly Gly Gly Leu Val Asp Val Gln Arg Arg Arg Ala Ala His Gly
 225 230 235 240
 Gly Glu Ala Pro Ala Ala His Arg Leu Leu Gln Pro Arg Arg Cys Arg
 245 250 255
 Ser Gly Gly Gln Gln Ala Ser Thr Arg Cys Ser Thr Arg Gin Thr
 260 265 270

<210> 22

<211> 156
 <212> PRT
 <213> Triticum aestivum

<400> 22
 Gln Arg Trp Thr Pro Ala Ala Ser Gly Ala Arg Arg Thr Arg Leu Val
 1 5 10 15
 Leu His Ser Ser Asp Arg Tyr Leu Cys Gly Ala Ile Val Leu Ala Gln
 20 25 30
 Ser Ile Arg Arg Ser Gly Ser Thr Arg Asp Met Val Leu Leu His Asp
 35 40 45
 His Thr Val Ser Lys Pro Ala Leu Arg Ala Leu Val Ala Ala Gly Trp
 50 55 60
 Ile Pro Arg Arg Ile Arg Arg Ile Arg Asn Pro Arg Ala Glu Arg Gly
 65 70 75 80
 Ser Tyr Asn Glu Tyr Asn Tyr Ser Lys Phe Arg Leu Trp Gln Leu Thr
 85 90 95
 Glu Tyr Phe Arg Val Val Phe Ile Asp Ala Asp Ile Leu Val Leu Arg
 100 105 110
 Ser Leu Asp Ala Leu Phe Arg Phe Pro Gln Ile Ser Ala Gly Gly Asn
 115 120 125
 Asp Gly Ser Leu Phe Asn Ser Gly Asn Met Val Leu Glu Pro Ser Ala
 130 135 140
 Cys Thr Phe Glu Ala Leu Val Arg Gly Arg Arg Thr
 145 150 155

<210> 23
 <211> 2028
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 <213> Arabidopsis thaliana

<220>
 <221> CDS
 <222> (1)...(1854)

<400> 23
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 Met Ile Pro Ser Ser Pro Met Glu Ser Arg His Arg Leu Ser Phe
 1 5 10 15
 tca aat gag aag aca agt agg agg aga ttt caa aga att gag aag ggt 96
 Ser Asn Glu Lys Thr Ser Arg Arg Phe Gln Arg Ile Glu Lys Gly
 20 25 30
 gtc aag ttc aac act ctg aaa ctt gtg ttg att tgt ata atg ctt gga 144
 Val Lys Phe Asn Thr Leu Lys Leu Val Leu Ile Cys Ile Met Leu Gly
 35 40 45
 gct ttg ttc acg atc tac cgt ttt cgt tat cca ccg cta caa att cct 192
 Ala Leu Phe Thr Ile Tyr Arg Phe Arg Tyr Pro Pro Leu Gln Ile Pro

39

| | | | |
|---|-----|-----|-----|
| 50 | 55 | 60 | |
| gaa att cca act agt ttt ggt ctt act act gat cct cgc tat gta gct | | | 240 |
| Glu Ile Pro Thr Ser Phe Gly Leu Thr Thr Asp Pro Arg Tyr Val Ala | | | |
| 65 | 70 | 75 | 80 |
| aca gct gag atc aac tgg aac cat atg tca aat ctt gtt gag aag cac | | | 288 |
| Thr Ala Glu Ile Asn Trp Asn His Met Ser Asn Leu Val Glu Lys His | | | |
| 85 | 90 | 95 | |
| gta ttt ggt aga agc gag tat caa gga att ggt ctt ata aat ctt aac | | | 336 |
| Val Phe Gly Arg Ser Glu Tyr Gln Gly Ile Gly Leu Ile Asn Leu Asn | | | |
| 100 | 105 | 110 | |
| gat aac gag att gat cga ttc aag gag gta acg aaa tct gac tgt gat | | | 384 |
| Asp Asn Glu Ile Asp Arg Phe Lys Glu Val Thr Lys Ser Asp Cys Asp | | | |
| 115 | 120 | 125 | |
| cat gta gct ttg cat cta gat tat gct gca aag aac ata aca tgg gaa | | | 432 |
| His Val Ala Leu His Leu Asp Tyr Ala Ala Lys Asn Ile Thr Trp Glu | | | |
| 130 | 135 | 140 | |
| tct tta tac ccg gaa tgg att gat gaa gtt gaa gaa ttc gaa gtc cct | | | 480 |
| Ser Leu Tyr Pro Glu Trp Ile Asp Glu Val Glu Glu Phe Glu Val Pro | | | |
| 145 | 150 | 155 | 160 |
| act tgt cct tct ctg cct ttg att caa att cct ggc aag cct cgg att | | | 528 |
| Thr Cys Pro Ser Leu Pro Leu Ile Gln Ile Pro Gly Lys Pro Arg Ile | | | |
| 165 | 170 | 175 | |
| gat ctt gta att gcc aag ctt ccg tgt gat aaa tca gga aaa tgg tct | | | 576 |
| Asp Leu Val Ile Ala Lys Leu Pro Cys Asp Lys Ser Gly Lys Trp Ser | | | |
| 180 | 185 | 190 | |
| aga gat gtg gct cgc ttg cat tta caa ctt gca gca gct cga gtg gcg | | | 624 |
| Arg Asp Val Ala Arg Leu His Leu Gln Leu Ala Ala Arg Val Ala | | | |
| 195 | 200 | 205 | |
| gct tct tct aaa gga ctt cat aat gtt cat gtg att ttg gta tct gat | | | 672 |
| Ala Ser Ser Lys Gly Leu His Asn Val His Val Ile Leu Val Ser Asp | | | |
| 210 | 215 | 220 | |
| tgc ttt cca ata ccg aat ctt ttt acg ggt caa gaa ctt gtt gcc cgt | | | 720 |
| Cys Phe Pro Ile Pro Asn Leu Phe Thr Gly Gln Glu Leu Val Ala Arg | | | |
| 225 | 230 | 235 | 240 |
| caa gga aac ata tgg ctg tat aag cct aat ctt cac cag cta aga caa | | | 768 |
| Gln Gly Asn Ile Trp Leu Tyr Lys Pro Asn Leu His Gln Leu Arg Gln | | | |
| 245 | 250 | 255 | |
| aag tta cag ctt cct gtt ggt tcc tgt gaa ctt tct gtt cct ctt caa | | | 816 |
| Lys Leu Gln Leu Pro Val Gly Ser Cys Glu Leu Ser Val Pro Leu Gln | | | |
| 260 | 265 | 270 | |

| | |
|---|------|
| gct aaa gat aat ttc tac tcc gca ggt gca aag aaa gaa gct tac gcg | 864 |
| Ala Lys Asp Asn Phe Tyr Ser Ala Gly Ala Lys Lys Glu Ala Tyr Ala | |
| 275 280 285 | |
| act atc ttg cat tct gcc caa ttt tat gtc tgt gga gcc att gca gct | 912 |
| Thr Ile Leu His Ser Ala Gln Phe Tyr Val Cys Gly Ala Ile Ala Ala | |
| 290 295 300 | |
| gca cag agc att cga atg tca ggc tct act cgt gat ctg gtc ata ctt | 960 |
| Ala Gln Ser Ile Arg Met Ser Gly Ser Thr Arg Asp Leu Val Ile Leu | |
| 305 310 315 320 | |
| gtt gat gaa acg ata agc gaa tac cat aaa agt ggc ttg gta gct gct | 1008 |
| Val Asp Glu Thr Ile Ser Glu Tyr His Lys Ser Gly Leu Val Ala Ala | |
| 325 330 335 | |
| gga tgg aag att caa atg ttt caa aga atc agg aac ccg aat gct gta | 1056 |
| Gly Trp Lys Ile Gln Met Phe Gln Arg Ile Arg Asn Pro Asn Ala Val | |
| 340 345 350 | |
| cca aat gcc tac aac gaa tgg aac tac agc aag ttt cgt ctt tgg caa | 1104 |
| Pro Asn Ala Tyr Asn Glu Trp Asn Tyr Ser Lys Phe Arg Leu Trp Gln | |
| 355 360 365 | |
| ctg act gaa tac agt aag atc atc ttc atc gat gca gac atg ctt atc | 1152 |
| Leu Thr Glu Tyr Ser Lys Ile Ile Phe Ile Asp Ala Asp Met Leu Ile | |
| 370 375 380 | |
| ctg aga aac att gat ttc ctc ttc gag ttc cct gag ata tca gca act | 1200 |
| Leu Arg Asn Ile Asp Phe Leu Phe Glu Phe Pro Glu Ile Ser Ala Thr | |
| 385 390 395 400 | |
| gga aac aat gct acg ctc ttc aac tct ggt cta atg gtg gtt gag cca | 1248 |
| Gly Asn Asn Ala Thr Leu Phe Asn Ser Gly Leu Met Val Val Glu Pro | |
| 405 410 415 | |
| tct aat tca aca ttc cag tta cta atg gat aac att aat gaa gtt gtg | 1296 |
| Ser Asn Ser Thr Phe Gln Leu Leu Met Asp Asn Ile Asn Glu Val Val | |
| 420 425 430 | |
| tct tac aac gga gga gac caa ggt tac ctt aac gag ata ttc aca tgg | 1344 |
| Ser Tyr Asn Gly Gly Asp Gln Gly Tyr Leu Asn Glu Ile Phe Thr Trp | |
| 435 440 445 | |
| tgg cat cgg att cca aaa cac atg aat ttc ttg aag cat ttc tgg gaa | 1392 |
| Trp His Arg Ile Pro Lys His Met Asn Phe Leu Lys His Phe Trp Glu | |
| 450 455 460 | |
| gga gac gaa cct gag att aaa aaa atg aag acg agt cta ttt gga gct | 1440 |
| Gly Asp Glu Pro Glu Ile Lys Lys Met Lys Thr Ser Leu Phe Gly Ala | |
| 465 470 475 480 | |

gat cct ccg atc cta tac gtt ctt cat tac cta ggt tat aac aaa ccc 1488
 Asp Pro Pro Ile Leu Tyr Val Leu His Tyr Leu Gly Tyr Asn Lys Pro
 485 490 495

 tgg tta tgc ttc aga gac tat gac tgc aat tgg aat gtc gat att ttc 1536
 Trp Leu Cys Phe Arg Asp Tyr Asp Cys Asn Trp Asn Val Asp Ile Phe
 500 505 510

 cag gaa ttt gct agt gac gag gct cat aaa acc tgg tgg aga gtg cac 1584
 Gln Glu Phe Ala Ser Asp Glu Ala His Lys Thr Trp Trp Arg Val His
 515 520 525

 gac gca atg cct gaa aac ttg cat aag ttc tgt cta cta aga tcg aaa 1632
 Asp Ala Met Pro Glu Asn Leu His Lys Phe Cys Leu Leu Arg Ser Lys
 530 535 540

 cag aag gcg caa ctt gaa tgg gat agg aga caa gca gag aaa ggg aac 1680
 Gln Lys Ala Gln Leu Glu Trp Asp Arg Arg Gln Ala Glu Lys Gly Asn
 545 550 555 560

 tac aaa gat gga cat tgg aag ata aag atc aaa gac aag aga ctt aag 1728
 Tyr Lys Asp Gly His Trp Lys Ile Lys Ile Lys Asp Lys Arg Leu Lys
 565 570 575

 act tgt ttc gaa gat ttc tgc ttt tgg gag agt atg ctt tgg cat tgg 1776
 Thr Cys Phe Glu Asp Phe Cys Phe Trp Glu Ser Met Leu Trp His Trp
 580 585 590

 ggt gag acg aac tct acc aac aat tct tcc acc acc acc act tca tca 1824
 Gly Glu Thr Asn Ser Thr Asn Asn Ser Ser Thr Thr Thr Ser Ser
 595 600 605

 ccg ccg cat aaa acc gct ctc cct tcc ctg tgaattcttt tggctttctg 1874
 Pro Pro His Lys Thr Ala Leu Pro Ser Leu
 610 615

 gtttggtaca aattactctg ccttcgcca accaaatgtg gtttggatat gttctttgt 1934

 ttttttatta tcagcttgaa acctgtatac gaatcccaga aacaatgtaa tcatgaggggg 1994

 ataaaggaat gaaagacaaa taaagaattt acag 2028

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 <211> 618
 <212> PRT
 <213> *Arabidopsis thaliana*

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 1 5 10 15

Ser Asn Glu Lys Thr Ser Arg Arg Arg Phe Gln Arg Ile Glu Lys Gly
20 25 30

Val Lys Phe Asn Thr Leu Lys Leu Val Leu Ile Cys Ile Met Leu Gly
35 40 45

Ala Leu Phe Thr Ile Tyr Arg Phe Arg Tyr Pro Pro Leu Gln Ile Pro
50 55 60

Glu Ile Pro Thr Ser Phe Gly Leu Thr Thr Asp Pro Arg Tyr Val Ala
65 70 75 80

Thr Ala Glu Ile Asn Trp Asn His Met Ser Asn Leu Val Glu Lys His
85 90 95

Val Phe Gly Arg Ser Glu Tyr Gln Gly Ile Gly Leu Ile Asn Leu Asn
100 105 110

Asp Asn Glu Ile Asp Arg Phe Lys Glu Val Thr Lys Ser Asp Cys Asp
115 120 125

His Val Ala Leu His Leu Asp Tyr Ala Ala Lys Asn Ile Thr Trp Glu
130 135 140

Ser Leu Tyr Pro Glu Trp Ile Asp Glu Val Glu Glu Phe Glu Val Pro
145 150 155 160

Thr Cys Pro Ser Leu Pro Leu Ile Gln Ile Pro Gly Lys Pro Arg Ile
165 170 175

Asp Leu Val Ile Ala Lys Leu Pro Cys Asp Lys Ser Gly Lys Trp Ser
180 185 190

Arg Asp Val Ala Arg Leu His Leu Gln Leu Ala Ala Ala Arg Val Ala
195 200 205

Ala Ser Ser Lys Gly Leu His Asn Val His Val Ile Leu Val Ser Asp
210 215 220

Cys Phe Pro Ile Pro Asn Leu Phe Thr Gly Gln Glu Leu Val Ala Arg
225 230 235 240

Gln Gly Asn Ile Trp Leu Tyr Lys Pro Asn Leu His Gln Leu Arg Gln
245 250 255

Lys Leu Gln Leu Pro Val Gly Ser Cys Glu Leu Ser Val Pro Leu Gln
260 265 270

Ala Lys Asp Asn Phe Tyr Ser Ala Gly Ala Lys Lys Glu Ala Tyr Ala
275 280 285

43

Thr Ile Leu His Ser Ala Gln Phe Tyr Val Cys Gly Ala Ile Ala Ala
290 295 300

Ala Gln Ser Ile Arg Met Ser Gly Ser Thr Arg Asp Leu Val Ile Leu
305 310 315 320

Val Asp Glu Thr Ile Ser Glu Tyr His Lys Ser Gly Leu Val Ala Ala
325 330 335

Gly Trp Lys Ile Gln Met Phe Gln Arg Ile Arg Asn Pro Asn Ala Val
340 345 350

Pro Asn Ala Tyr Asn Glu Trp Asn Tyr Ser Lys Phe Arg Leu Trp Gln
355 360 365

Leu Thr Glu Tyr Ser Lys Ile Ile Phe Ile Asp Ala Asp Met Leu Ile
370 375 380

Leu Arg Asn Ile Asp Phe Leu Phe Glu Phe Pro Glu Ile Ser Ala Thr
385 390 395 400

Gly Asn Asn Ala Thr Leu Phe Asn Ser Gly Leu Met Val Val Glu Pro
405 410 415

Ser Asn Ser Thr Phe Gln Leu Leu Met Asp Asn Ile Asn Glu Val Val
420 425 430

Ser Tyr Asn Gly Gly Asp Gln Gly Tyr Leu Asn Glu Ile Phe Thr Trp
435 440 445

Trp His Arg Ile Pro Lys His Met Asn Phe Leu Lys His Phe Trp Glu
450 455 460

Gly Asp Glu Pro Glu Ile Lys Lys Met Lys Thr Ser Leu Phe Gly Ala
465 470 475 480

Asp Pro Pro Ile Leu Tyr Val Leu His Tyr Leu Gly Tyr Asn Lys Pro
485 490 495

Trp Leu Cys Phe Arg Asp Tyr Asp Cys Asn Trp Asn Val Asp Ile Phe
500 505 510

Gln Glu Phe Ala Ser Asp Glu Ala His Lys Thr Trp Trp Arg Val His
515 520 525

Asp Ala Met Pro Glu Asn Leu His Lys Phe Cys Leu Leu Arg Ser Lys
530 535 540

Gln Lys Ala Gln Leu Glu Trp Asp Arg Arg Gln Ala Glu Lys Gly Asn
545 550 555 560

Tyr Lys Asp Gly His Trp Lys Ile Lys Ile Lys Asp Lys Arg Leu Lys

| | | |
|---|-----------|-----|
| 565 | 44 570 | 575 |
| Thr Cys Phe Glu Asp Phe Cys Phe Trp Glu Ser Met Leu Trp His Trp | | |
| 580 | 585 | 590 |
| Gly Glu Thr Asn Ser Thr Asn Asn Ser Ser Thr Thr Thr Ser Ser | | |
| 595 | 600 | 605 |
| Pro Pro His Lys Thr Ala Leu Pro Ser Leu | | |
| 610 | 615 | |

<210> 25
 <211> 1845
 <212> DNA
 <213> Oryza sativa

<220>
 <221> CDS
 <222> (1)..(1845)

| | | |
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| <400> 25 | 48 | |
| atg ggg gtg acg ggc ggc ggg gag gcc gtc aag ccg tcg tcg tcg | | |
| Met Gly Val Thr Gly Gly Ala Gly Glu Ala Val Lys Pro Ser Ser Ser | | |
| 1 | 5 | 10 |
| | | 15 |
| tcg tcg ttg tcg ccg gtg gcg ggg ctg agg gcg gcg gcc atc gtg aag | 96 | |
| Ser Ser Leu Ser Pro Val Ala Gly Leu Arg Ala Ala Ala Ile Val Lys | | |
| 20 | 25 | 30 |
| ctg aac gcg gcg ttc ctc gcc ttc ttc ctc gcg tac atg gcg ctc | 144 | |
| Leu Asn Ala Ala Phe Leu Ala Phe Phe Phe Leu Ala Tyr Met Ala Leu | | |
| 35 | 40 | 45 |
| ctc ctc cac ccc aag tac tcc tac ctc ctc gac cgc ggc gcc gcc tcc | 192 | |
| Leu Leu His Pro Lys Tyr Ser Tyr Leu Leu Asp Arg Gly Ala Ala Ser | | |
| 50 | 55 | 60 |
| tcc ctc gtc cgc tgc acc gcc ttc cgc gac gcc tgc acc ccg gcg acg | 240 | |
| Ser Leu Val Arg Cys Thr Ala Phe Arg Asp Ala Cys Thr Pro Ala Thr | | |
| 65 | 70 | 75 |
| | | 80 |
| acg acc acc gcc cag ctc tct cgg aag ctg gga ggc gtg gcg gcg aac | 288 | |
| Thr Thr Thr Ala Gln Leu Ser Arg Lys Leu Gly Gly Val Ala Ala Asn | | |
| 85 | 90 | 95 |
| aag gcg gtg gcg gcg gcg gag agg atc gtg aac gcc ggg agg gcg | 336 | |
| Lys Ala Val Ala Ala Ala Glu Arg Ile Val Asn Ala Gly Arg Ala | | |
| 100 | 105 | 110 |
| ccg gcg atg ttc gac gag ctc cgt ggg cgg ctg cgg atg ggc ctg gtg | 384 | |

45

| | | | |
|---|------|-----|-----|
| Pro Ala Met Phe Asp Glu Leu Arg Gly Arg Leu Arg Met Gly Leu Val | | | |
| 115 | 120 | 125 | |
| | | | |
| aac atc ggc cgc gac gag ctg ctg gcg ctc ggc gtg gag ggc gac gcc | 432 | | |
| Asn Ile Gly Arg Asp Glu Leu Leu Ala Leu Gly Val Glu Gly Asp Ala | | | |
| 130 | 135 | 140 | |
| | | | |
| gtc ggc gtc gac ttc gag cgc gtc tcc gac atg ttc cgg tgg tcg gac | 480 | | |
| Val Gly Val Asp Phe Glu Arg Val Ser Asp Met Phe Arg Trp Ser Asp | | | |
| 145 | 150 | 155 | 160 |
| | | | |
| ctc ttc ccg gag tgg atc gac gag gag gac gac gag ggc ccg tcc | 528 | | |
| Leu Phe Pro Glu Trp Ile Asp Glu Glu Asp Asp Glu Gly Pro Ser | | | |
| 165 | 170 | 175 | |
| | | | |
| tgc ccg gag ctc ccc atg ccg gac ttc tcc cgg tac ggc gac gtc gac | 576 | | |
| Cys Pro Glu Leu Pro Met Pro Asp Phe Ser Arg Tyr Gly Asp Val Asp | | | |
| 180 | 185 | 190 | |
| | | | |
| gtg gtg gtg gcg ctg ccg tgc aac cgt tcg gac gcc gcg tgg aac | 624 | | |
| Val Val Val Ala Ser Leu Pro Cys Asn Arg Ser Asp Ala Ala Trp Asn | | | |
| 195 | 200 | 205 | |
| | | | |
| cgc gac gtg ttc agg ctg cag gtg cac ctc gtg acg gcg cac atg gcg | 672 | | |
| Arg Asp Val Phe Arg Leu Gln Val His Leu Val Thr Ala His Met Ala | | | |
| 210 | 215 | 220 | |
| | | | |
| gcg cgc aag ggg ctg cgg cac gac gcc ggc ggc ggc ggc ggc ggg | 720 | | |
| Ala Arg Lys Gly Leu Arg His Asp Ala Gly Gly Gly Gly Gly Gly | | | |
| 225 | 230 | 235 | 240 |
| | | | |
| cgg gtg cgc gtg gtg cgc agc gag tgc gag ccc atg atg gac ttg | 768 | | |
| Arg Val Arg Val Val Arg Ser Glu Cys Glu Pro Met Met Asp Leu | | | |
| 245 | 250 | 255 | |
| | | | |
| ttc cgg tgc gac gag gcg gtg ggg agg gac ggc gag tgg tgg atg tac | 816 | | |
| Phe Arg Cys Asp Glu Ala Val Gly Arg Asp Gly Glu Trp Trp Met Tyr | | | |
| 260 | 265 | 270 | |
| | | | |
| atg gtc gac gtc gag cgg ctg gag gag aag ctc cgg ctt cct gtc ggc | 864 | | |
| Met Val Asp Val Glu Arg Leu Glu Glu Lys Leu Arg Leu Pro Val Gly | | | |
| 275 | 280 | 285 | |
| | | | |
| tca tgc aac ctc gcc cta cct ctg tgg gga ccc gga ggt atc cag gaa | 912 | | |
| Ser Cys Asn Leu Ala Leu Pro Leu Trp Gly Pro Gly Gly Ile Gln Glu | | | |
| 290 | 295 | 300 | |
| | | | |
| gtg ttc aac gtg tcg gag ctg acg gcg gcg gca acg gcg ggg cgg | 960 | | |
| Val Phe Asn Val Ser Glu Leu Thr Ala Ala Ala Ala Thr Ala Gly Arg | | | |
| 305 | 310 | 315 | 320 |
| | | | |
| ccg cgg cgg gag gcg tac gcg acg gtg ctc cac tcg tac gac acg tac | 1008 | | |

46

| | | | |
|---|------|-----|-----|
| Pro Arg Arg Glu Ala Tyr Ala Thr Val Leu His Ser Ser Asp Thr Tyr | | | |
| 325 | 330 | 335 | |
| ctg tgc ggc gcg atc gtg ctg gcg cag agc atc cgg cgc gcc ggg tcg | 1056 | | |
| Leu Cys Gly Ala Ile Val Leu Ala Gln Ser Ile Arg Arg Ala Gly Ser | | | |
| 340 | 345 | 350 | |
| acg cgc gac ctc gtc ctc cac gac cac acc gtg tcg aag ccg gcg | 1104 | | |
| Thr Arg Asp Leu Val Leu Leu His Asp His Thr Val Ser Lys Pro Ala | | | |
| 355 | 360 | 365 | |
| ctg gcg gcg ctg gtc gcc ggc tgg acc ccg cgc aag atc aag cgc | 1152 | | |
| Leu Ala Ala Leu Val Ala Ala Gly Trp Thr Pro Arg Lys Ile Lys Arg | | | |
| 370 | 375 | 380 | |
| atc cgc aac ccg cgc gcg gag cgc ggc acc tac aac gag tac aac tac | 1200 | | |
| Ile Arg Asn Pro Arg Ala Glu Arg Gly Thr Tyr Asn Glu Tyr Asn Tyr | | | |
| 385 | 390 | 395 | 400 |
| acg aag ttc cgg ctg tgg cag ctc acc gac tac gac cgc gtg gtg ttc | 1248 | | |
| Ser Lys Phe Arg Leu Trp Gln Leu Thr Asp Tyr Asp Arg Val Val Phe | | | |
| 405 | 410 | 415 | |
| gtc gac gcc gac atc ctc gtc ctc cgc gac ctc gac gcc ctc ttc ggc | 1296 | | |
| Val Asp Ala Asp Ile Leu Val Leu Arg Asp Leu Asp Ala Leu Phe Gly | | | |
| 420 | 425 | 430 | |
| ttc ccg cag ctg acg gcg gtg ggc aac gac ggc tcg ctc ttc aac tcc | 1344 | | |
| Phe Pro Gln Leu Thr Ala Val Gly Asn Asp Gly Ser Leu Phe Asn Ser | | | |
| 435 | 440 | 445 | |
| ggg gtg atg gtg atc gag ccg tcg cag tgc acg ttc cag tcg ctg atc | 1392 | | |
| Gly Val Met Val Ile Glu Pro Ser Gln Cys Thr Phe Gln Ser Leu Ile | | | |
| 450 | 455 | 460 | |
| cgg cag cgg acc atc cgg tcc tac aac ggc ggc gat cag ggg ttc | 1440 | | |
| Arg Gln Arg Arg Thr Ile Arg Ser Tyr Asn Gly Gly Asp Gln Gly Phe | | | |
| 465 | 470 | 475 | 480 |
| ctg aac gag gtg ttc gtc tgg tgg cac cgg ctg ccg cgg cgg gtg aac | 1488 | | |
| Leu Asn Glu Val Phe Val Trp Trp His Arg Leu Pro Arg Arg Val Asn | | | |
| 485 | 490 | 495 | |
| tac ctc aag aac ttc tgg gcg aac act acg gcg gag cgg gcg ctc aag | 1536 | | |
| Tyr Leu Lys Asn Phe Trp Ala Asn Thr Thr Ala Glu Arg Ala Leu Lys | | | |
| 500 | 505 | 510 | |
| gag cgg ctg ttc cgg gcg gat ccc gcg gag gtg tgg tcg atc cac tac | 1584 | | |
| Glu Arg Leu Phe Arg Ala Asp Pro Ala Glu Val Trp Ser Ile His Tyr | | | |
| 515 | 520 | 525 | |
| ctg ggg ctg aag ccg tgg acg tgc tac ccg gac tac gac tgc aac tgg | 1632 | | |

47

Leu Gly Leu Lys Pro Trp Thr Cys Tyr Arg Asp Tyr Asp Cys Asn Trp
 530 535 540

aac atc ggc gac cag cgg gtg tac gcc agc gac gcc gcg cac gcg cgg 1680
 Asn Ile Gly Asp Gln Arg Val Tyr Ala Ser Asp Ala Ala His Ala Arg
 545 550 555 560

tgg tgg cag gtg tac gac gac atg ggg gag gcc atg cgc tcg ccg tgc 1728
 Trp Trp Gln Val Tyr Asp Asp Met Gly Glu Ala Met Arg Ser Pro Cys
 565 570 575

cgc ctg tcg gag cgg agg aag atc gag atc gcc tgg gac cga cac ctc 1776
 Arg Leu Ser Glu Arg Arg Lys Ile Glu Ile Ala Trp Asp Arg His Leu
 580 585 590

gcc gag gag gcc ggc ttc tcc gac cac cac tgg aag atc aac atc acc 1824
 Ala Glu Glu Ala Gly Phe Ser Asp His His Trp Lys Ile Asn Ile Thr
 595 600 605

gac ccc cgc aag tgg gag tag 1845
 Asp Pro Arg Lys Trp Glu *
 610

<210> 26

<211> 614

<212> PRT

<213> Oryza sativa

<400> 26

Met Gly Val Thr Gly Gly Ala Gly Glu Ala Val Lys Pro Ser Ser Ser
 1 5 10 15
 Ser Ser Leu Ser Pro Val Ala Gly Leu Arg Ala Ala Ala Ile Val Lys
 20 25 30
 Leu Asn Ala Ala Phe Leu Ala Phe Phe Leu Ala Tyr Met Ala Leu
 35 40 45
 Leu Leu His Pro Lys Tyr Ser Tyr Leu Leu Asp Arg Gly Ala Ala Ser
 50 55 60
 Ser Leu Val Arg Cys Thr Ala Phe Arg Asp Ala Cys Thr Pro Ala Thr
 65 70 75 80
 Thr Thr Thr Ala Gln Leu Ser Arg Lys Leu Gly Gly Val Ala Ala Asn
 85 90 95
 Lys Ala Val Ala Ala Ala Glu Arg Ile Val Asn Ala Gly Arg Ala
 100 105 110
 Pro Ala Met Phe Asp Glu Leu Arg Gly Arg Leu Arg Met Gly Leu Val
 115 120 125
 Asn Ile Gly Arg Asp Glu Leu Leu Ala Leu Gly Val Glu Gly Asp Ala
 130 135 140
 Val Gly Val Asp Phe Glu Arg Val Ser Asp Met Phe Arg Trp Ser Asp
 145 150 155 160
 Leu Phe Pro Glu Trp Ile Asp Glu Glu Asp Asp Glu Gly Pro Ser
 165 170 175

Cys Pro Glu Leu Pro Met Pro Asp Phe Ser Arg Tyr Gly Asp Val Asp
 180 185 190
 Val Val Val Ala Ser Leu Pro Cys Asn Arg Ser Asp Ala Ala Trp Asn
 195 200 205
 Arg Asp Val Phe Arg Leu Gln Val His Leu Val Thr Ala His Met Ala
 210 215 220
 Ala Arg Lys Gly Leu Arg His Asp Ala Gly Gly Gly Gly Gly Gly
 225 230 235 240
 Arg Val Arg Val Val Arg Ser Glu Cys Glu Pro Met Met Asp Leu
 245 250 255
 Phe Arg Cys Asp Glu Ala Val Gly Arg Asp Gly Glu Trp Trp Met Tyr
 260 265 270
 Met Val Asp Val Glu Arg Leu Glu Glu Lys Leu Arg Leu Pro Val Gly
 275 280 285
 Ser Cys Asn Leu Ala Leu Pro Leu Trp Gly Pro Gly Gly Ile Gln Glu
 290 295 300
 Val Phe Asn Val Ser Glu Leu Thr Ala Ala Ala Ala Thr Ala Gly Arg
 305 310 315 320
 Pro Arg Arg Glu Ala Tyr Ala Thr Val Leu His Ser Ser Asp Thr Tyr
 325 330 335
 Leu Cys Gly Ala Ile Val Leu Ala Gln Ser Ile Arg Arg Ala Gly Ser
 340 345 350
 Thr Arg Asp Leu Val Leu Leu His Asp His Thr Val Ser Lys Pro Ala
 355 360 365
 Leu Ala Ala Leu Val Ala Ala Gly Trp Thr Pro Arg Lys Ile Lys Arg
 370 375 380
 Ile Arg Asn Pro Arg Ala Glu Arg Gly Thr Tyr Asn Glu Tyr Asn Tyr
 385 390 395 400
 Ser Lys Phe Arg Leu Trp Gln Leu Thr Asp Tyr Asp Arg Val Val Phe
 405 410 415
 Val Asp Ala Asp Ile Leu Val Leu Arg Asp Leu Asp Ala Leu Phe Gly
 420 425 430
 Phe Pro Gln Leu Thr Ala Val Gly Asn Asp Gly Ser Leu Phe Asn Ser
 435 440 445
 Gly Val Met Val Ile Glu Pro Ser Gln Cys Thr Phe Gln Ser Leu Ile
 450 455 460
 Arg Gln Arg Arg Thr Ile Arg Ser Tyr Asn Gly Gly Asp Gln Gly Phe
 465 470 475 480
 Leu Asn Glu Val Phe Val Trp Trp His Arg Leu Pro Arg Arg Val Asn
 485 490 495
 Tyr Leu Lys Asn Phe Trp Ala Asn Thr Thr Ala Glu Arg Ala Leu Lys
 500 505 510
 Glu Arg Leu Phe Arg Ala Asp Pro Ala Glu Val Trp Ser Ile His Tyr
 515 520 525
 Leu Gly Leu Lys Pro Trp Thr Cys Tyr Arg Asp Tyr Asp Cys Asn Trp
 530 535 540
 Asn Ile Gly Asp Gln Arg Val Tyr Ala Ser Asp Ala Ala His Ala Arg
 545 550 555 560
 Trp Trp Gln Val Tyr Asp Asp Met Gly Glu Ala Met Arg Ser Pro Cys
 565 570 575
 Arg Leu Ser Glu Arg Arg Lys Ile Glu Ile Ala Trp Asp Arg His Leu
 580 585 590

49

Ala Glu Glu Ala Gly Phe Ser Asp His His Trp Lys Ile Asn Ile Thr
 595 600 605
 Asp Pro Arg Lys Trp Glu
 610

<210> 27
 <211> 626
 <212> DNA
 <213> Zea mays

<220>
 <221> CDS
 <222> (133) .. (624)

<400> 27
 ttcgagcggc cgcccccggc aggtacaaac ctgacgtgaa ggctctaaag gagaagctca 60
 ggctgcctgt tggttcctgt gagcttgctg ttccactcaa cgcaaaagca cgactcttac 120
 acggtagaca ga cgc aga gaa gca tat gct aca ata ctt cat tca gca agt 171
 Arg Arg Glu Ala Tyr Ala Thr Ile Leu His Ser Ala Ser
 1 5 10
 gaa tat gtt tgc ggt gcg ata aca gca gct caa agc att cgt caa gca 219
 Glu Tyr Val Cys Gly Ala Ile Thr Ala Ala Gln Ser Ile Arg Gln Ala
 15 20 25
 gga tca aca aga gac ctt gtt att ctt gtt gat gac acc ata agt gac 267
 Gly Ser Thr Arg Asp Leu Val Ile Leu Val Asp Asp Thr Ile Ser Asp
 30 35 40 45
 cac cac cgc aag ggg ctg gaa tct gct ggg tgg aag gtc aga ata ata 315
 His His Arg Lys Gly Leu Glu Ser Ala Gly Trp Lys Val Arg Ile Ile
 50 55 60
 gaa agg atc cgg aat ccc aaa gcc gaa cgt gat gcc tac aac gaa tgg 363
 Glu Arg Ile Arg Asn Pro Lys Ala Glu Arg Asp Ala Tyr Asn Glu Trp
 65 70 75
 aac tac agc aaa ttc cgg ctg tgg cag ctt aca gat tac gac aag gtt 411
 Asn Tyr Ser Lys Phe Arg Leu Trp Gln Leu Thr Asp Tyr Asp Lys Val
 80 85 90
 att ttc att gat gct gat ctg ctc atc ctg agg aac att gat ttc ttg 459
 Ile Phe Ile Asp Ala Asp Leu Leu Arg Asn Ile Asp Phe Leu
 95 100 105
 ttt gca atg cca gaa atc acc gca act ggg aac aat gcc aca ctc ttc 507
 Phe Ala Met Pro Glu Ile Thr Ala Thr Gly Asn Asn Ala Thr Leu Phe
 110 115 120 125

50

| | | |
|---|-----|-----|
| aac tct ggg gtc atg gtc att'gaa cct tca aac tgc acg ttc cag tta | 555 | |
| Asn Ser Gly Val Met Val Ile Glu Pro Ser Asn Cys Thr Phe Gln Leu | | |
| 130 | 135 | 140 |
| ctg atg gag cac atc aac gag ata aca tct tac aac ggt ggt gac caa | 603 | |
| Leu Met Glu His Ile Asn Glu Ile Thr Ser Tyr Asn Gly Gly Asp Gln | | |
| 145 | 150 | 155 |
| ggg tac ctc ggc cgc gac cac gc | 626 | |
| Gly Tyr Leu Gly Arg Asp His | | |
| 160 | | |

<210> 28
<211> 164
<212> PRT
<213> Zea mays

| | | | |
|---|-----|-----|-----|
| <400> 28 | | | |
| Arg Arg Glu Ala Tyr Ala Thr Ile Leu His Ser Ala Ser Glu Tyr Val | | | |
| 1 | 5 | 10 | 15 |
| Cys Gly Ala Ile Thr Ala Ala Gln Ser Ile Arg Gln Ala Gly Ser Thr | | | |
| 20 | 25 | 30 | |
| Arg Asp Leu Val Ile Leu Val Asp Asp Thr Ile Ser Asp His His Arg | | | |
| 35 | 40 | 45 | |
| Lys Gly Leu Glu Ser Ala Gly Trp Lys Val Arg Ile Ile Glu Arg Ile | | | |
| 50 | 55 | 60 | |
| Arg Asn Pro Lys Ala Glu Arg Asp Ala Tyr Asn Glu Trp Asn Tyr Ser | | | |
| 65 | 70 | 75 | 80 |
| Lys Phe Arg Leu Trp Gln Leu Thr Asp Tyr Asp Lys Val Ile Phe Ile | | | |
| 85 | 90 | 95 | |
| Asp Ala Asp Leu Leu Ile Leu Arg Asn Ile Asp Phe Leu Phe Ala Met | | | |
| 100 | 105 | 110 | |
| Pro Glu Ile Thr Ala Thr Gly Asn Asn Ala Thr Leu Phe Asn Ser Gly | | | |
| 115 | 120 | 125 | |
| Val Met Val Ile Glu Pro Ser Asn Cys Thr Phe Gln Leu Leu Met Glu | | | |
| 130 | 135 | 140 | |
| His Ile Asn Glu Ile Thr Ser Tyr Asn Gly Gly Asp Gln Gly Tyr Leu | | | |
| 145 | 150 | 155 | 160 |
| Gly Arg Asp His | | | |

<210> 29
 <211> 553
 <212> DNA
 <213> Zea mays

<220>
 <221> CDS
 <222> (1) .. (552)

<400> 29 48
 tgg aag gtc aga ata ata gaa agg atc cgg aat ccc aaa gcc gaa cgt Trp Lys Val Arg Ile Ile Glu Arg Ile Arg Asn Pro Lys Ala Glu Arg
 1 5 10 15
 gat gcc tac aac gaa tgg aac tac agc aaa ttc cgg ctg tgg cag ctt 96
 Asp Ala Tyr Asn Glu Trp Asn Tyr Ser Lys Phe Arg Leu Trp Gln Leu
 20 25 30
 aca gat tac gac aag gtt att ttc att gat gct gat ctg ctc atc ctg 144
 Thr Asp Tyr Asp Lys Val Ile Phe Ile Asp Ala Asp Leu Leu Ile Leu
 35 40 45
 agg aac att gat ttc ttg ttt gca atg cca gaa atc acc gca act ggg 192
 Arg Asn Ile Asp Phe Leu Phe Ala Met Pro Glu Ile Thr Ala Thr Gly
 50 55 60
 aac aat gcc aca ctc ttc aac tct ggg gtg atg gtc att gaa cct tca 240
 Asn Asn Ala Thr Leu Phe Asn Ser Gly Val Met Val Ile Glu Pro Ser
 65 70 75 80
 aac tgc acg ttc cag tta ctg atg gag cac atc aac gag ata aca tct 288
 Asn Cys Thr Phe Gln Leu Leu Met Glu His Ile Asn Glu Ile Thr Ser
 85 90 95
 tac aac ggt ggt gac caa ggg tac ctg aac gag ata ttc aca tgg tgg 336
 Tyr Asn Gly Gly Asp Gln Gly Tyr Leu Asn Glu Ile Phe Thr Trp Trp
 100 105 110
 cac cgg att cca aag cac atg aat ttc ttg aag cat ttc tgg gag ggt 384
 His Arg Ile Pro Lys His Met Asn Phe Leu Lys His Phe Trp Glu Gly
 115 120 125
 gat gag gac gaa gtg aag gcc aag aag act cgg ctg ttc ggc gcc aac 432
 Asp Glu Asp Glu Val Lys Ala Lys Lys Thr Arg Leu Phe Gly Ala Asn
 130 135 140
 cca ccg atc ctc tac gtt ctc cac tac ttg ggg cgg aag cca tgg ctg 480
 Pro Pro Ile Leu Tyr Val Leu His Tyr Leu Gly Arg Lys Pro Trp Leu
 145 150 155 160

52

tgc ttc cg^g gac tac gat tgc aac tgg aac gtc gag atc ttg cg^g gag 528
 Cys Phe Arg Asp Tyr Asp Cys Asn Trp Asn Val Glu Ile Leu Arg Glu
 165 170 175

ttt gc^g agt gac gtt gc^g cat gc^c 553
 Phe Ala Ser Asp Val Ala His Ala
 180

<210> 30
 <211> 184
 <212> PRT
 <213> Zea mays

<400> 30
 Trp Lys Val Arg Ile Ile Glu Arg Ile Arg Asn Pro Lys Ala Glu Arg
 1 5 10 15

Asp Ala Tyr Asn Glu Trp Asn Tyr Ser Lys Phe Arg Leu Trp Gln Leu
 20 25 30

Thr Asp Tyr Asp Lys Val Ile Phe Ile Asp Ala Asp Leu Leu Ile Leu
 35 40 45

Arg Asn Ile Asp Phe Leu Phe Ala Met Pro Glu Ile Thr Ala Thr Gly
 50 55 60

Asn Asn Ala Thr Leu Phe Asn Ser Gly Val Met Val Ile Glu Pro Ser
 65 70 75 80

Asn Cys Thr Phe Gln Leu Leu Met Glu His Ile Asn Glu Ile Thr Ser
 85 90 95

Tyr Asn Gly Gly Asp Gln Gly Tyr Leu Asn Glu Ile Phe Thr Trp Trp
 100 105 110

His Arg Ile Pro Lys His Met Asn Phe Leu Lys His Phe Trp Glu Gly
 115 120 125

Asp Glu Asp Glu Val Lys Ala Lys Lys Thr Arg Leu Phe Gly Ala Asn
 130 135 140

Pro Pro Ile Leu Tyr Val Leu His Tyr Leu Gly Arg Lys Pro Trp Leu
 145 150 155 160

Cys Phe Arg Asp Tyr Asp Cys Asn Trp Asn Val Glu Ile Leu Arg Glu
 165 170 175

Phe Ala Ser Asp Val Ala His Ala
 180

<210> 31
 <211> 552
 <212> DNA
 <213> Zea mays

<220>
 <221> CDS
 <222> (1)..(552)

<400> 31 48
 tcc ctg cgc cgg ctc agc ccc aac gcc gac cgc gtc gtc atc gcg tcc Ser Leu Arg Arg Leu Ser Pro Asn Ala Asp Arg Val Val Ile Ala Ser 1 5 10 15
 ctc gac gtc ccg ccg ctc tgg gtt cag gca ctg aaa aat gac ggg gta Leu Asp Val Pro Pro Leu Trp Val Gln Ala Leu Lys Asn Asp Gly Val 20 25 30
 aag gtg gtc tct gtg gag aat ttg aaa aat cct tac gag aaa caa gaa Lys Val Val Ser Val Glu Asn Leu Lys Asn Pro Tyr Glu Lys Gln Glu 35 40 45
 aat ttc aac aga cga ttc aaa ttg act tta aac aag ctg tat gca tgg Asn Phe Asn Arg Arg Phe Lys Leu Thr Leu Asn Lys Leu Tyr Ala Trp 50 55 60
 agc ttg gtt tca tat gag cga gtt gtt atg ctt gac tct gac aac att Ser Leu Val Ser Tyr Glu Arg Val Val Met Leu Asp Ser Asp Asn Ile 65 70 75 80
 ttc ctc caa aat act gat gag tta ttt cag tgt ggt cag ttc tgt gct Phe Leu Gln Asn Thr Asp Glu Leu Phe Gln Cys Gly Gln Phe Cys Ala 85 90 95
 gtc ttc atc aat ccc tgt atc ttc cat aca ggt ctt ttt gtg ctt cag Val Phe Ile Asn Pro Cys Ile Phe His Thr Gly Leu Phe Val Leu Gln 100 105 110
 ccc tca atg gat gtt ttt aag aac atg cta cat gag cta gcg gtt gga Pro Ser Met Asp Val Phe Lys Asn Met Leu His Glu Leu Ala Val Gly 115 120 125
 cgt gaa aac cca gat ggg gca gac caa ggc ttc ctt gct agt tat ttc Arg Glu Asn Pro Asp Gly Ala Asp Gln Gly Phe Leu Ala Ser Tyr Phe 130 135 140
 ccg gac ttg ctt gat cag cca atg ttc cat cca cca gct aat ggt aca Pro Asp Leu Leu Asp Gln Pro Met Phe His Pro Pro Ala Asn Gly Thr 145 150 155 160
 aaa ctt tgg ggt act tat cgc ctc ccc cta ggc tac cag atg gat gca 528

54

Lys Leu Trp Gly Thr Tyr Arg Leu Pro Leu Gly Tyr Gln Met Asp Ala
 165 170 175

tct tac tat tat ctg aag ctt cgc
 Ser Tyr Tyr Tyr Leu Lys Leu Arg
 180

552

<210> 32
 <211> 184
 <212> PRT
 <213> Zea mays

<400> 32
 Ser Leu Arg Arg Leu Ser Pro Asn Ala Asp Arg Val Val Ile Ala Ser
 1 5 10 15

Leu Asp Val Pro Pro Leu Trp Val Gln Ala Leu Lys Asn Asp Gly Val
 20 25 30

Lys Val Val Ser Val Glu Asn Leu Lys Asn Pro Tyr Glu Lys Gln Glu
 35 40 45

Asn Phe Asn Arg Arg Phe Lys Leu Thr Leu Asn Lys Leu Tyr Ala Trp
 50 55 60

Ser Leu Val Ser Tyr Glu Arg Val Val Met Leu Asp Ser Asp Asn Ile
 65 70 75 80

Phe Leu Gln Asn Thr Asp Glu Leu Phe Gln Cys Gly Gln Phe Cys Ala
 85 90 95

Val Phe Ile Asn Pro Cys Ile Phe His Thr Gly Leu Phe Val Leu Gln
 100 105 110

Pro Ser Met Asp Val Phe Lys Asn Met Leu His Glu Leu Ala Val Gly
 115 120 125

Arg Glu Asn Pro Asp Gly Ala Asp Gln Gly Phe Leu Ala Ser Tyr Phe
 130 135 140

Pro Asp Leu Leu Asp Gln Pro Met Phe His Pro Pro Ala Asn Gly Thr
 145 150 155 160

Lys Leu Trp Gly Thr Tyr Arg Leu Pro Leu Gly Tyr Gln Met Asp Ala
 165 170 175

Ser Tyr Tyr Tyr Leu Lys Leu Arg
 180

<210> 33
 <211> 560
 <212> DNA
 <213> Zea mays

<220>
 <221> CDS
 <222> (1) .. (558)

<400> 33

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|---|-----|
| aaa cct gac gtg aag gcg ttg aag gag aag ctc agg ctg cct gtt ggt | 48 |
| Lys Pro Asp Val Lys Ala Leu Lys Glu Lys Leu Arg Leu Pro Val Gly | |
| 1 | 5 |
| | 10 |
| | 15 |
| | |
| tcc tgt gag ctt gct gtt cca ctc aac gca aaa gca cga ctc tac aca | 96 |
| Ser Cys Glu Leu Ala Val Pro Leu Asn Ala Lys Ala Arg Leu Tyr Thr | |
| 20 | 25 |
| | 30 |
| | |
| gta gac aga cgc aga gaa gca tat gcg aca ata ctg cat tca gca agt | 144 |
| Val Asp Arg Arg Glu Ala Tyr Ala Thr Ile Leu His Ser Ala Ser | |
| 35 | 40 |
| | 45 |
| | |
| gaa tat gtt tgc ggc gcg atc acg gca gct caa agc att cgt caa gca | 192 |
| Glu Tyr Val Cys Gly Ala Ile Thr Ala Ala Gln Ser Ile Arg Gln Ala | |
| 50 | 55 |
| | 60 |
| | |
| gga tca aca aga gac ctc gtt att ctc gtc gac gac acc ata agt gac | 240 |
| Gly Ser Thr Arg Asp Leu Val Ile Leu Val Asp Asp Thr Ile Ser Asp | |
| 65 | 70 |
| | 75 |
| | 80 |
| | |
| cac cac cgc aag ggg ctg caa tct gcg ggg tgg aag gtc agg ata ata | 288 |
| His His Arg Lys Gly Leu Gln Ser Ala Gly Trp Lys Val Arg Ile Ile | |
| 85 | 90 |
| | 95 |
| | |
| cag agg atc cgg aac ccc aaa gcc gag cgc gac gcc tac aac gag tgg | 336 |
| Gln Arg Ile Arg Asn Pro Lys Ala Glu Arg Asp Ala Tyr Asn Glu Trp | |
| 100 | 105 |
| | 110 |
| | |
| aac tac agc aaa ttc cgg ctg tgg cag ctc acg gat tac gac aag gtc | 384 |
| Asn Tyr Ser Lys Phe Arg Leu Trp Gln Leu Thr Asp Tyr Asp Lys Val | |
| 115 | 120 |
| | 125 |
| | |
| atc ttc atc gac gcg gat ctc ctc atc ctg agg aac atc gat ttc ctg | 432 |
| Ile Phe Ile Asp Ala Asp Leu Ile Leu Arg Asn Ile Asp Phe Leu | |
| 130 | 135 |
| | 140 |
| | |
| ttc gcg ctg ccg gag atc acg gcg acg ggg aac aac gcg acg ctc ttc | 480 |
| Phe Ala Leu Pro Glu Ile Thr Ala Thr Gly Asn Asn Ala Thr Leu Phe | |
| 145 | 150 |
| | 155 |
| | 160 |
| | |
| aac tcg gga gtg atg gtc atc gag cct tcg aac tgc acg ttc cgg cta | 528 |
| Asn Ser Gly Val Met Val Ile Glu Pro Ser Asn Cys Thr Phe Arg Leu | |

| | | | | |
|---|----|-----|-----|---|
| 165 | 56 | 170 | 175 | |
| | | | | 560 |
| ctg atg gag cac atc gac gag ata acg tcg ta | | | | |
| Leu Met Glu His Ile Asp Glu Ile Thr Ser | | | | |
| 180 185 | | | | |
| | | | | |
| <210> 34 | | | | |
| <211> 186 | | | | |
| <212> PRT | | | | |
| <213> Zea mays | | | | |
| | | | | |
| <400> 34 | | | | |
| Lys Pro Asp Val Lys Ala Leu Lys Glu Lys Leu Arg Leu Pro Val Gly | | | | |
| 1 | 5 | 10 | 15 | |
| | | | | |
| Ser Cys Glu Leu Ala Val Pro Leu Asn Ala Lys Ala Arg Leu Tyr Thr | | | | |
| 20 25 | | | | 30 |
| | | | | |
| Val Asp Arg Arg Arg Glu Ala Tyr Ala Thr Ile Leu His Ser Ala Ser | | | | |
| 35 40 | | | | 45 |
| | | | | |
| Glu Tyr Val Cys Gly Ala Ile Thr Ala Ala Gln Ser Ile Arg Gln Ala | | | | |
| 50 55 | | | | 60 |
| | | | | |
| Gly Ser Thr Arg Asp Leu Val Ile Leu Val Asp Asp Thr Ile Ser Asp | | | | |
| 65 70 | | | | 75 80 |
| | | | | |
| His His Arg Lys Gly Leu Gln Ser Ala Gly Trp Lys Val Arg Ile Ile | | | | |
| 85 90 | | | | 95 |
| | | | | |
| Gln Arg Ile Arg Asn Pro Lys Ala Glu Arg Asp Ala Tyr Asn Glu Trp | | | | |
| 100 105 | | | | 110 |
| | | | | |
| Asn Tyr Ser Lys Phe Arg Leu Trp Gln Leu Thr Asp Tyr Asp Lys Val | | | | |
| 115 120 | | | | 125 |
| | | | | |
| Ile Phe Ile Asp Ala Asp Leu Leu Ile Leu Arg Asn Ile Asp Phe Leu | | | | |
| 130 135 | | | | 140 |
| | | | | |
| Phe Ala Leu Pro Glu Ile Thr Ala Thr Gly Asn Asn Ala Thr Leu Phe | | | | |
| 145 150 | | | | 155 160 |
| | | | | |
| Asn Ser Gly Val Met Val Ile Glu Pro Ser Asn Cys Thr Phe Arg Leu | | | | |
| 165 170 | | | | 175 |
| | | | | |
| Leu Met Glu His Ile Asp Glu Ile Thr Ser | | | | |
| 180 185 | | | | |

<210> 35

<211> 566

<212> PRT

<213> Arabidopsis thaliana

<400> 35

Met Gly Ala Lys Ser Lys Ser Ser Ser Thr Arg Phe Phe Met Phe Tyr
1 5 10 15

Leu Ile Leu Ile Ser Leu Ser Phe Leu Gly Leu Leu Leu Asn Phe Lys
20 25 30

Pro Leu Phe Leu Leu Asn Pro Met Ile Ala Ser Pro Ser Ile Val Glu
35 40 45

Ile Arg Tyr Ser Leu Pro Glu Pro Val Lys Arg Thr Pro Ile Trp Leu
50 55 60

Arg Leu Ile Arg Asn Tyr Leu Pro Asp Glu Lys Lys Ile Arg Val Gly
65 70 75 80

Leu Leu Asn Ile Ala Glu Asn Glu Arg Glu Ser Tyr Glu Ala Ser Gly
85 90 95

Thr Ser Ile Leu Glu Asn Val His Val Ser Leu Asp Pro Leu Pro Asn
100 105 110

Asn Leu Thr Trp Thr Ser Leu Phe Pro Val Trp Ile Asp Glu Asp His
115 120 125

Thr Trp His Ile Pro Ser Cys Pro Glu Val Pro Leu Pro Lys Met Glu
130 135 140

Gly Ser Glu Ala Asp Val Asp Val Val Val Lys Val Pro Cys Asp
145 150 155 160

Gly Phe Ser Glu Lys Arg Gly Leu Arg Asp Val Phe Arg Leu Gln Val
165 170 175

Asn Leu Ala Ala Ala Asn Leu Val Val Glu Ser Gly Arg Arg Asn Val
180 185 190

Asp Arg Thr Val Tyr Val Val Phe Ile Gly Ser Cys Gly Pro Met His

195 200 205
Glu Ile Phe Arg Cys Asp Glu Arg Val Lys Arg Val Gly Asp Tyr Trp
210 215 220

Val Tyr Arg Pro Asp Leu Thr Arg Leu Lys Gln Lys Leu Leu Met Pro
225 230 235 240

Pro Gly Ser Cys Gln Ile Ala Pro Leu Gly Gln Gly Glu Ala Trp Ile
245 250 255

58

Gln Asp Lys Asn Arg Asn Leu Thr Ser Glu Lys Thr Thr Leu Ser Ser
260 265 270

Phe Thr Ala Gln Arg Val Ala Tyr Val Thr Leu Leu His Ser Ser Glu
275 280 285

Val Tyr Val Cys Gly Ala Ile Ala Leu Ala Gln Ser Ile Arg Gln Ser
290 295 300

Gly Ser Thr Lys Asp Met Ile Leu Leu His Asp Asp Ser Ile Thr Asn
305 310 315 320

Ile Ser Leu Ile Gly Leu Ser Leu Ala Gly Trp Lys Leu Arg Arg Val
325 330 335

Glu Arg Ile Arg Ser Pro Phe Ser Lys Lys Arg Ser Tyr Asn Glu Trp
340 345 350

Asn Tyr Ser Lys Leu Arg Val Trp Gln Val Thr Asp Tyr Asp Lys Leu
355 360 365

Val Phe Ile Asp Ala Asp Phe Ile Ile Val Lys Asn Ile Asp Tyr Leu
370 375 380

Phe Ser Tyr Pro Gln Leu Ser Ala Ala Gly Asn Asn Lys Val Leu Phe
385 390 395 400

Asn Ser Gly Val Met Val Leu Glu Pro Ser Ala Cys Leu Phe Glu Asp
405 410 415

Leu Met Leu Lys Ser Phe Lys Ile Gly Ser Tyr Asn Gly Asp Gln
420 425 430

Gly Phe Leu Asn Glu Tyr Phe Val Trp Trp His Arg Leu Ser Lys Arg
435 440 445

Leu Asn Thr Met Lys Tyr Phe Gly Asp Glu Ser Arg His Asp Lys Ala
450 455 460

Arg Asn Leu Pro Glu Asn Leu Glu Gly Ile His Tyr Leu Gly Leu Lys
465 470 475 480

Pro Trp Arg Cys Tyr Arg Asp Tyr Asp Cys Asn Trp Asp Leu Lys Thr
485 490 495

Arg Arg Val Tyr Ala Ser Glu Ser Val His Ala Arg Trp Trp Lys Val
500 505 510

Tyr Asp Lys Met Pro Lys Lys Leu Lys Gly Tyr Cys Gly Leu Asn Leu
515 520 525

Lys Met Glu Lys Asn Val Glu Lys Trp Arg Lys Met Ala Lys Leu Asn

59

530

535

540

Gly Phe Pro Glu Asn His Trp Lys Ile Arg Ile Lys Asp Pro Arg Lys
545 550 555 560

Lys Asn Arg Leu Ser Glu
565